# Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

# U. S. DEPARTMENT OF AGRICULTURE

FARMERS' BULLETIN No. 214.

# BENEFICIAL BACTERIA FOR LEGUMINOUS CROPS.

BY

#### GEORGE T. MOORE,

Physiotogist and Algologist, In Charge of Laboratory of Plant Physiology,

AND

#### T. R. ROBINSON,

Assistant in Physiotogy, Vegetable Pathological and Physiological Investigations, Bureau of Plant Industry.



# WASHINGTON: GOVERNMENT PRINTING OFFICE. 1905.

# LETTER OF TRANSMITTAL

U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. OFFICE OF THE CHIEF, Washington, D. C., January 17, 1905.

SIR: I have the honor to transmit herewith a paper on Beneficial Bacteria for Leguminous Crops, and to recommend that it be published as a Farmers' Bulletin. This paper was prepared by Dr. George T. Moore, in charge of the Laboratory of Plant Physiology, and Mr. T. R. Robinson, Assistant in Physiology in the Office of Vegetable Pathological and Physiological Investigations, and was submitted by Mr. A. F. Woods, the Pathologist and Physiologist, with a view to publication.

Special attention is called to the fact that there is no monopoly in the methods and processes set forth in this paper. Recognizing the vast importance to the farmers of the country of an unrestricted use of the nitrogen-fixing organisms described, a patent was taken out for the Government in the name of Doetor Moore. The object of the patent is to prevent any monopoly in the manufacture of the material. the manufacture and sale of the nitrogen-fixing organisms are free to any reliable firm in the United States or elsewhere, the Department will not hesitate to eall attention to any attempt to deceive farmers in this matter, either by the offering of spurious material or material at prices which the Department knows by its experience to be exorbitant. This action is necessary to protect the farmers for whose direct benefit the entire work is designed.

I greatly regret to state that the supply of packages for inoculating legumes is completely exhausted for this season. Applications for material for use next spring should not be made before September 1. 1905.

Respectfully,

B. T. GALLOWAY, Chief of Bureau.

Hon. JAMES WILSON, Secretary of Agriculture.

# CONTENTS.

	Page.
Introduction	5
Nitrogen, the all-important element in crop production	5
The position in agriculture of leguminous crops	7
Effect of root nodules upon growth and yield	7
Nitrogen fixation as affecting succeeding crops	8
Artificial inoculation of the soil.	9
Soil transfer	9
Invention and use of "nitragin".	10
Formation of root nodules	11
Cross-inoculation	12
Influence of climatic and soil conditions	13
Infection without forming root nodules	14
Improved methods for growing and distributing nodule-forming bacteria	15
Use of nitrogen-free media	15
Dry cultures	15
Liquid cultures	16
Time of inoculation	17
Preparing liquid culture for field use	19
Applying the liquid culture	19
Use of fertilizers in connection with inoculation	22
Choice of legume for green manuring	24
When should the farmer resort to artificial inoculation?	27
When inoculation is necessary	27
When inoculation is desirable	28
When inoculation is worthy of trial	28
When inoculation is unnecessary	28
When to expect failure with inoculation	29
Circular of information to farmers	30
Field tests by practical farmers	31
Distribution of inoculating material	31
Reports showing proportion of successes and failures	32
Some characteristic experiences	33
Alfalfa	34
Red clover	37
Cowpeas	39
Garden peas	40
Beans	42
Soy beans	43
Hairy vetch	44
Crimson clover.	44
Sweet peas	45
Canada field peas	45
Velvet beans.	46
Berseem	46
Peanuts	46
Miscellaneous	46
Summary	47

# ILLUSTRATIONS.

			Page.
Fig.	1.	Roots of young alfalfa plants showing nodules	11
	2.	Roots of melilotus (sweet clover) showing nodules	11
	3.	Roots of crimson clover showing nodules	12
		Roots of red clover showing nodules	16
	5.	Roots of soy bean showing nodules	17
	6.	Roots of hairy vetch showing nodules	18
		Roots of velvet bean showing nodules	_ 19
		Roots of sweet pea showing nodules	21
		Roots of lupine showing nodules	22
	10.	Roots of sainfoin showing nodules	23
	11.	Roots of garden pea showing nodules	24
	12.	A few cells from a lupine nodule, magnified 1,000 times to show the bacteria	25
	13.	Branching forms of bacteria from a clover nodule; magnified 2,000 times	25
	14.	Rod forms of bacteria from a fenugreek nodule; magnified 2,000 times.	25
	15.	Clouding of culture liquid (in flask on left) due to the growth of bacteria in forty-eight hours	26
	16.	Sprinkling Canada field pea seed with culture liquid; method employed on a large fruit farm in California	26
	17.	Stirring seed moistened with culture liquid to hasten drying	27

# BENEFICIAL BACTERIA FOR LEGUMINOUS CROPS."

#### INTRODUCTION.

The part which leguminous crops play in maintaining soil fertility has long been a matter of speculation among practical farmers and a subject for critical investigation on the part of scientific men. Plants of this family, the Leguminosæ (which, in general, bear their seeds in a pod or legume), when grown in contact with certain bacteria form upon their roots small knots or nodules variously known as "nitrogen knots" or "nitrogen traps" from the part they play in furnishing to the plants nitrogen derived from the air. The primary object in undertaking an investigation of the fixation of nitrogen by these root nodules was to devise, if possible, some method of bringing about the artificial introduction of the necessary organisms into soils which were naturally devoid of them, and at the same time to attempt, as far as possible, to correlate and reconcile the vast amount of conflicting evidence that has been accumulated by various investigators in regard to the exact nature of the organism, where the nitrogen is fixed, the effect upon the host plant, and similar problems.

# NITROGEN, THE ALL-IMPORTANT ELEMENT IN CROP PRODUCTION.

Ever since anything has been known in regard to plant nutrition and the necessary part that various gases and minerals play in the successful growing of crops, scientific men have realized the great importance of conserving the world's store of nitrogen, and have made every effort either to husband or to increase all available sources of supply. In the early days, when it was first realized that nitrogen was so essential to plant life—in fact, was at the very foundation of agriculture—no

<sup>&</sup>lt;sup>a</sup> The very great demand for information on the subject of nitrogen fixation by soil inoculation has made it desirable to prepare this general account of the subject with special reference to the needs of farmers. The article on Bacteria and the Nitrogen Problem in the Yearbook for 1902 is now no longer available for distribution, but a bulletin (No. 71 of the Bureau of Plant Industry) which treats more at length of this subject can be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., by sending 15 cents in currency or postal money order.—A. F. Woods, Pathologist and Physiologist.

particular alarm was felt. Botanists had demonstrated that plants obtained their earbon from the carbon dioxid of the air, and since this gas is present in so much less quantity than nitrogen it was believed that by no possible means could the most essential of plant foods be exhausted. However, when it was shown that plants were unable to use free atmospheric nitrogen and must obtain it directly from the soil in a highly organized form, the importance of the problem increased greatly, and the gravest consequences were predicted by those familiar with the rapidity with which this valuable element was being wasted.

But a short time ago Sir William Crookes predicted that within thirty or forty years England would experience a wheat famine, due to the exhaustion of nitrogen of the soil, that would be appalling in its effect; and Prof. Bela Korasey's warnings to Hungary have been even more emphatic. Indeed, Liebig, more than fifty years ago, in speaking of one of the most common methods of destroying sources of available nitrogen, said:

Nothing will more certainly consummate the ruin of England than the scarcity of fertilizers. It means the scarcity of food. It is impossible that such a sinful violation of the divine laws of nature should forever remain unpunished, and the time will probably come for England, sooner than for any other country, when, with all her wealth in gold, iron, and coal, she will be unable to buy the one-thousandth part of the food which she has during hundreds of years thrown recklessly away.

The ways by which combined nitrogen is rendered unavailable for plant food are well known and need no elaborate discussion. The constant cropping of land, combined with our modern sewage system, which prevents the return to the soil of such a large and legitimate nitrogen supply, are sufficient to indicate the extent of this loss without considering the destruction of nitrogenous compounds by the denitrifying bacteria, the burning or exploding of nitrate of soda, and the leaching out of this and other salts which would otherwise be most valuable as fertilizers.

These things would not merit so much consideration were it not for the fact that, unfortunately, the world's supply of two of the richest sources of nitrogen—guano and saltpeter—is being exhausted rapidly. Guano has already ceased to be of any great importance, and while it is difficult to obtain precise estimates as to the available amount of saltpeter, it is very certain that, at the present rate of its consumption (estimated at 1,000,000,000 tons a year) it can not last for a very great length of time, some placing the limit at less than fifty years. It should also be remembered that the natural product, while so rich in nitrogen, is also so expensive that for the general farmer the cost is often almost prohibitive. The same may be said of the process recently proposed for the manufacture of nitrogen salts by means of electricity. While the discovery and perfection of such a method are

calculated to calm the fears of those who predict a nitrogen famine, it is not one that appeals very strongly to the farmer so long as the price remains where it is.

#### THE POSITION IN AGRICULTURE OF LEGUMINOUS CROPS.

From the earliest days of agriculture it has been recognized that all plants belonging to the Leguminosæ had a decidedly beneficial effect upon the soil. Pliny wrote: "The bean ranks first among the legumes. It fertilizes the ground in which it has been sown as well as any manure." And again: "The lupine enriches the soil of a field or vineyard as well as the very best manure. The vetch, too, enriches the soil and requires no attention in its culture." Varro, in De Re Rustica, I, 23, writes: "Legumes should be sown in light soils; indeed, they are planted not so much for their own crop as for the following crop, since when they are cut and kept upon the ground they make the soil better. Thus the lupine is wont to serve as a manure where the soil is rather thin and poor." There are also in ancient writings many other references to the importance and necessity of including some leguminous crop in the regular rotation. Naturally, the explanations offered to account for this beneficial effect were various, perhaps the most universal belief being that the root system of these plants was much more extensive than that of grains and root crops, and consequently brought up plant food from considerable depths, which not only served the legume, but was likewise available for subsequent crops.

During the past century various theories have been advanced to explain the growth of legumes in soils nearly or quite deficient in nitrogen, and the benefit to such soils from their growth, but it was not until 1886 that Helriegel announced at a scientific meeting that the source of nitrogen for these plants was unquestionably the atmosphere. Two years later, together with Willfarth, he demonstrated the fact that the growth of plants in soil free from nitrogen always occurred after the development of nodules or swellings upon the roots. Later the results obtained by these two men were fully substantiated by many other investigators, and the explanation of the long unsolved

problem was made possible.

# EFFECT OF ROOT NODULES UPON GROWTH AND YIELD.

The actual benefit of the presence of root nodules upon various leguminous plants has been so thoroughly demonstrated by numerous observers, both in this country and abroad, that it hardly needs further proof at this time. The early work of Helriegel and Willfarth, together with that of Lawes and Gilbert and of Warington in England, and of Atwater and Woods in this country, was quite sufficient to demonstrate the direct connection between the acquisition of nitrogen

in some way by the plant and the presence of the tuber-like swellings on its roots; and there are few, if any, who would maintain that this peculiar function is not, under most circumstances, distinctly beneficial. Indeed, by using clean sand, burned free of all organic matter, it is possible to demonstrate beyond question that leguminous plants will make a more vigorous growth when furnished with nitrogen by nodule bacteria than when it is supplied as fertilizer and nodules are absent. In pot experiments with vetch the inoculated plants exceeded nearly three times by weight those receiving nitrogenous fertilizer (6.16 grams and 2.65 grams, respectively), while plants having no nitrogen supplied them were insignificant in size (weight, 0.33 gram). With the exception of the nitrogen element, the nutrient solution used in watering the pots was the same in all three cases. Similar results have been obtained in the field.

G. L. Thomas, experimenting with field peas on his farm near Auburn, Me., made a special test with fertilized and unfertilized strips, and stated that "inoculated seed did as much without fertilizers of any kind as seed not inoculated but supplied fertilizer (phosphate) at the rate of 800 pounds and a ton of barnyard manure per acre."

With garden peas, S. N. Lowry, of Philadelphia, found that "inoculated vines yielded one and a half the crop yielded from ground not inoculated, but which was manured;" and Jeremiah Gardner, of Gaffney, S. C., wrote: "My cowpeas were better than those of others who used commercial fertilizer. They ripened early and evenly. I consider inoculation a boon to agriculture."

Federico Narro, of Saltillo, Mexico, noted the following result with alfalfa:

I have seen the progress of the inoculated alfalfa in the fields of J. Garcia Rodriguez, and, although failure was expected by the people who knew that he was going to sow alfalfa without manure, it is already coming out nicely and more even than the alfalfa sown in land covered with 8 or 10 inches of manure.

## NITROGEN FIXATION AS AFFECTING SUCCEEDING CROPS.

A convincing demonstration of the effect upon a soil of a leguminous erop bearing root nodules is to note the striking differences between crops of grain or vegetables that follow legumes and a similar erop grown on fallow land, or following a grass or vegetable erop. This is a matter of such common observation that a rotation including some leguminous erop is the practice of every farmer who has a thought for maintaining the fertility of his soil. It is easily proved that part of this benefit is due to the amount of nitrogen fixed by the bacteria of the root nodules and not to the unusual length of the root system or other peculiarities of the plant. Careful experiments to establish this fact have been earried on by many of the experiment stations in the United States, the results all tending to prove that a leguminous crop

affects the soil which bears it like the addition of a considerable amount of nitrogenous fertilizer. Averaging results obtained in sixteen States, the amount of nitrogen added per acre has been computed as 122 pounds; in Germany it is estimated at 200 pounds per acre.

When it is remembered that a high grade of nitrate of soda contains only about 15 per cent of nitrogen, while much that is on the market contains considerably less, it will be seen that a crop of nodule-bearing legumes is equal to from 800 to 1,000 pounds of nitrate of soda per acre, which at the present rate for this fertilizer represents a value of from \$20 to \$25.

#### ARTIFICIAL INOCULATION OF THE SOIL.

Since the desirability of introducing a leguminous crop into rotation seems to be of such importance, and the benefits to be obtained from a nodule-bearing plant are so evident, it is not surprising that every effort has been made to obtain crops which possess the power of using atmospheric nitrogen. It has been found, however, that, although in a great many instances the organisms producing nodules are naturally abundant in the soil, and the mere planting of the regume seed is sufficient to produce a crop capable of fixing nitrogen, there are also some localities which are devoid of the necessary bacteria, and in such places the seed sown either fails to make a stand or the crop, drawing its nitrogen from the soil, is of no more benefit to the soil than a cereal or other "main crop" whose yield might be a distinct source of profit. Under these circumstances the attempt to grow the leguminous crop is a clear waste of time and labor unless proper steps are taken to introduce into the soil the organisms adapted to furnish the plant with atmospheric nitrogen.

#### SOIL TRANSFER.

To devise some means of artificially introducing into the soil the nodule-producing organisms has become necessary, and naturally the simplest means of accomplishing this has been to transfer earth known to contain the proper organisms, and capable of producing nodules, to the fields where it was desired to introduce such bacteria. This soil-transfer method is one which has been practiced widely, both in this country and abroad, oftentimes with the best results, but not with universal success. Reports have been received from various places stating that even where soil known to contain the proper germs was used the results were not satisfactory. That this failure was not due to the character of the soil or other adverse conditions is proved by the success of other methods of inoculation upon the same kind of land at the same time. The large quantity of earth necessary to produce thorough inoculation often makes it a laborious and expensive process when the fields to be treated are at a considerable distance.

In addition to the expense and labor involved, however, there is a more serious objection because of the possibility of transferring plant diseases from one field to another.

H. C. Coesten, of Walnut, Kans., reports having transplanted the "leaf blight" to his field by this method, and many instances are known in the South of the wilt of cowpeas being disseminated by carrying soil from one field to another. There can be no doubt that certain diseases of plants, the spores of which remain in the earth, may be widely disseminated by attempting to produce inoculation by the transfer of soil; and where the disease is one which causes great damage to leguminous crops and is readily transported, it has become necessary to abandon inoculation altogether.

There is also great danger of introducing objectionable weeds whereever soil from one locality is introduced into another region. Even though the weeds may not have been serious in the first field, the great numbers of dormant seeds which often require but the slightest change in environment to produce germination are always a menace, and a number of instances have been reported to the Department of Agriculture where the desired leguminous crop was completely choked out by the introduced weed. The director of the Mississippi Experiment Station writes: "Owing to the fact that our alfalfa fields are more or less full of Johnson grass, we are unable to send soil for the purpose of inoculation without distributing this objectionable grass to sections where the farmers are trying to keep it out."

#### INVENTION AND USE OF "NITRAGIN."

In order to escape the difficulties previously mentioned, a German botanist, Professor Nobbe, of Tharandt, eonceived the idea of bringing about inoculation by means of pure cultures. This was to be accomplished by isolating from the nodule the right organisms and then transferring them to tubes or bottles containing a nutrient jelly as a basis for growth. To this culture of nodule-forming bacteria was given the trade name of "nitragin." Seventeen different kinds of nitragin were prepared from the nodules of as many different plants, and arrangements were made to have them put up on a large scale and placed upon the market by a well-known firm of manufacturing chemists.

Experiments with nitragin in Germany met with varying degrees of success. In some instances its use seemed to produce an abundant formation of nodules, while in other cases no benefit could be obtained. In this country the results obtained by Duggar were very satisfactory, but other investigators were not able to secure inoculation. The chief difficulties seem to have been in securing cultures of the proper degree of virulence, and in preventing deterioration because of being subjected to too much heat or to varying degrees of moisture. The age of the culture was also of importance, the manufacturers limiting the time of efficiency of the culture to about six weeks.

Owing probably to the difficulty of maintaining the efficiency of the culture and the adverse conditions to which it was often subjected during transportation, the percentage of failures in the use of nitragin was so great that its manufacture was given up, and it is no longer for sale under that name. Consequently, even though this preparation had been found satisfactory in Europe, the necessity for devising some method of producing nitrogen-fixing nodules free from the objectionable features of transferring soil remained. For this reason the Laboratory of Plant Physiology of the Department of Agriculture undertook a scientific investigation of the root-nodule organism with a view to making practicable for use in the United States the pureculture method of inoculation.

#### FORMATION OF ROOT NODULES.

Before any improvement upon methods already in use could be hoped for, it was necessary to become thoroughly acquainted with the

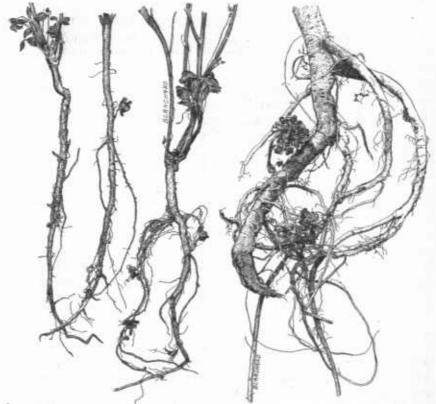


Fig. 1.—Roots of young alfalfa plants showing nodules.

Fig. 2.—Roots of melilotus (sweet clover) showing nodules.

precise nature of the nodule-forming organism, for, in spite of the fact that these organisms occur in great numbers and that the interior of the nodule constitutes what is practically a pure culture, there has

been the widest difference of opinion as to the character of these bodies.

One reason for the different theories in regard to the true cause of the legume nodule has undoubtedly been the diverse forms assumed

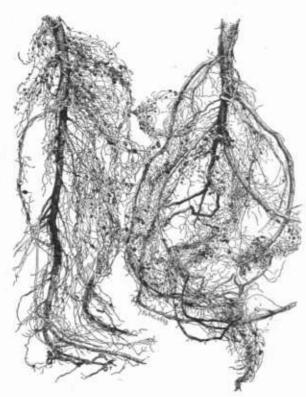


Fig. 3.—Roots of crimson clover showing nodules.

by the organisms found in the nodule at different times and under different conditions. This diversity of form has given rise to opinions classifying them variously as "funguslike" bodies, true fungi, yeasts, "bacteria-like" bodies. and true bacteria. (See figs. 12, 13, and 14.) They are properly to be regarded as bacteria, though differing in some minor characters from the majority of this group. They gain admission to the plant through the root hairs and require but a short time to increase

greatly in number. The irritation thus caused in the root tissue results in the rootlet taking an altered form; that is, a nodule is formed. These nodules vary from minute spheres to swellings as large as marbles, while the lobed, fiattened forms sometimes occur in clusters much larger. (See figs. 1 to 11.)

#### CROSS-INOCULATION.

Because of the fact frequently observed that one kind of legume would not produce nodules in soil which abundantly supplied another legume with these growths it has been supposed that each legume required a special and peculiar nodule organism. Efforts have been made to distinguish between these bacteria specifically, and separate names have been assigned to the microbes from the nodules of peas, beans, clover, etc. A study of the organisms involved reveals the fact that no real differences exist. Pot experiments with sixteen

different legumes, using a pure culture of the nodule organisms from one of the series, have shown that it is possible to cause the formation of nodules upon practically all legumes, no matter what the source of the organisms. The organisms used in such cross-inoculation must, however, first undergo cultivation upon an artificial medium deprived of combined nitrogen.

The wide differences shown by bacteria (as occurring in soils) in their ability to infect various leguminous crops are due no doubt to long adaptation to the peculiar reactions afforded by the host plant. For this reason specific cultures will usually produce more abundant nodules in a given time than a fixed or universal culture, although grown upon nitrogen-free media. The practical import of this is that it will be necessary, in order to obtain the best results, to prepare specific cultures for specific crops. For this reason the farmer, in procuring cultures, should always furnish definite information as to the kind of clover, peas, etc., which he wishes to inoculate.

## INFLUENCE OF CLIMATIC AND SOIL CONDITIONS.

Moisture.—The readiness with which root nodules are formed varies widely with the degree of moisture in the soil. Experiments by Gain and others have shown that with peas, beans, and lupines, watered and unwatered, the number of nodules in moist soil exceeded those in dry soil from ten to twenty times, and experiments in this country have demonstrated most conclusively that the humidity of a soil greatly favors nodule formation. This fact must be due either to the inability of the organism to come in contact with the root hairs in the absence of sufficient moisture or to a failure to penetrate the root hairs under such conditions, for drought is in no way fatal to the bacteria.

Air.—In artificial cultures it was conclusively shown that the organisms deprived of air soon perish, the nitrogen of the air especially being essential to growth. The importance of thorough working of the soil to permit the free entrance of air becomes at once apparent. Securing a proper degree of tilth has the added benefit of conserving the soil moisture.

Light.—Direct sunlight is injurious to the growing organisms, but probably has little effect when they are once introduced into the soil and covered by harrowing. No degree of cold will produce death, though multiplication is practically stopped at 10° C. (50° F.).

Acidity.—The importance of neutralizing the acidity of certain soils in order to be successful in growing clover, alfalfa, etc., is well known, and the addition of lime is frequently recommended where such crops fail. In such cases it is probable that the acidity of the soil not only is prejudicial to the growth of the plant, but has likewise prevented the development of the nodule-forming bacteria. Thus the addition of the lime serves a double purpose.

Clover sickness.—Much of the so-called "clover sickness" is probably due to the accumulation of acidity through continual cropping. The organisms, once effective, have deteriorated and no longer render assistance to the plants. In addition to liming, therefore, it will often be found advantageous in such regions to introduce a fresh supply of bacteria by artificial inoculation. This applies to a large portion of the castern and central portions of the United States. Failūres to secure inoculation with cultures sent out during the past year have, in a large number of the cases investigated, been traceable to a decidedly acid soil reaction. The importance of this factor can not be too strongly emphasized, especially as the cure is, in most cases, easy of application and inexpensive.

Testing soil for acidity.—Whether the soil is acid or not can be readily learned by bringing a strip of blue litmus paper (obtainable at any drug store) in contact with moist soil and allowing it to remain for twenty-four hours; a change from blue to pink (or pinkish) will take place if the soil is acid. Lime <sup>a</sup> (applied as ground limestone, or air-slaked burnt lime) at the rate of 1,000 to 1,500 pounds per acre will usually be sufficient for a first application. It should be evenly distributed and well worked into the soil before attempting inoculation.

For special soils, however, there is reason to believe that by artificial methods of growth adaptations of the bacteria may be brought about, so that special cultures to suit such soils will be secured.

Nitrates.—The fact that the nodules do not occur abundantly upon plants growing in very rich earth has been frequently observed. Not only does the available nitrogen in the soil render the activity of the bacteria less essential to healthy plant growth, but the presence of nitrogenous substances seems to have a distinctly unfavorable effect upon the bacteria themselves. Aside from the proof of this fact shown in artificial cultures it has been strikingly illustrated in field practice, using the same culture to inoculate plants growing in rich, medium, and poor soils. C. E. Jones, of Carysbrook, Va., in reporting upon the inoculation of soy beans, furnished three lots of specimen plants, saying, "No. 1, from the poorer places, an excessive growth of nodules; No. 2, from loose sand and soil, quite numerous but smaller; No. 3, from a rich, loamy spot, not so abundant (few in fact), and some plants having no nodules at all."

#### INFECTION WITHOUT FORMING ROOT NODULES.

The result of certain experiments with cultures sent out from the Department of Agriculture indicated that in these cases at least there was a decided benefit from inoculation although no nodules appeared. With soy beans the plants so inoculated made a decidedly better growth

a See Farmers' Bulletin, No. 77, U. S. Dept. of Agriculture, "The Liming of Soils."

than nodule-bearing plants inoculated with soil from an old field. The check plants (not inoculated) were much inferior to both. The tests were carried on at one of the State experiment stations and careful observations were made.

Similar results were obtained with alfalfa, berscen, and kidney beans in other localities, widely separated. Examination under the microscope of specimens secured showed in each case that the smaller roots were infected with the bacteria usually occurring in nodules, and these bacteria were thus in a new way assisting the plants by their power of fixing nitrogen. Cases of this character must be more fully investigated before any conclusion as to the factors involved can be reached.

## IMPROVED METHODS FOR GROWING AND DISTRIBUTING NODULE-FORMING BACTERIA.

The method of growth was found to have a determining influence upon the virulence and keeping qualities of the cultures. Reference has already been made to nitragin; its liability to deterioration and the lack of virulence of the organisms were factors which made this product a failure in field practice. The medium used for inducing growth was a nutrient jelly, the basis of which was an extract of legume stems and roots. This gave a rapid growth of the bacteria, but contained such a ready store of combined nitrogen that the organisms were not forced to utilize the nitrogen of the air and as a consequence soon lost this valuable power. Overfed, they became inert and failed to produce nodules when brought in contact with the host plant.

### USE OF NITROGEN-FREE MEDIA.

To overcome this difficulty numerous combinations of nutrient salts were tried and it was found that growth would take place in a solution lacking nitrogen, and further that the organisms thus produced preserved their ability to fix nitrogen and form nodules to a degree hitherto unknown. Bacteria grown upon media of this character proved to be much more virulent than those cultivated on a rich nitrogenous base, and experiments on a large scale showed the greatest difference in the nodule-producing power of organisms grown by these two methods.

#### DRY CULTURES.

How to maintain the necessary degree of virulence now became a problem of first importance in securing practical results. Even though the efficiency of the culture be at its highest point, the mere fact of its having to grow for a considerable time under artificial conditions is apt to weaken it; consequently, the means of preserving and distributing the bacteria after they are propagated becomes fully as

important as the method of obtaining them in sufficient quantity for such distribution. This is another reason why the German product sent out upon rich nutrient media failed to maintain its original strength, and if it had not been possible to devise some more satisfactory way of delivering cultures to the farmer it is probable that little success could ever have been attained by the pure-culture method.



Fig. 4.—Roots of red clover showing nodules.

The method which has been employed in the Department of Agriculture for the past year has been to saturate absorbent eotton in a liquid eulture of the noduleforming organism. In this way millions of the baeteria are held within the cotton, and after this is carefully dried out they remain dormant in much the same way as seeds, waiting for the proper conditions to revive them.

#### LIQUID CULTURES.

The "dry eulture" thus produced needs only to be immersed in water to start the organisms into growth. To hasten the growth, how-

ever, it seemed best to furnish with the cotton culture two packages of nutrient salts, one containing sugar, magnesium sulphate, and potassium phosphate, and the other ammonium phosphate. By the addition of the first three ingredients to the water a solution is formed which is not well adapted for the growth of the organisms usually carried about in the air (yeasts, molds, etc.), but is well suited for the multiplication of the nodule-forming bacteria. The addition of the ammonium phosphate at the end of twenty-four hours tends to increase still further the growth of these bacteria, which are already well

started if the temperature has not been too low or too high. With the food thus supplied, a period of two days gives a growth sufficient to change clear water to a milky liquid, ready to treat seed or soil. (See fig. 15.)

Process patented.—The method being perfected, it was deemed advisable that a patent should be taken out, thus securing for the use of the Department of Agriculture the result of its own investigations

and guaranteeing to the public that no monopoly could be maintained by commercial producers of similar cultures. Under the provisions of the patent, however, no restriction is placed upon the manufacture of efficient and properly prepared cultures by such concerns or individuals.

#### TIME OF INOCU-LATION.

It has generally been accepted as true that to secure an effective inoculation the organisms must be brought into contact with the plants at an early stage of growth.



Fig. 5.-Roots of soy bean showing nodules.

Laboratory experiments have indicated that after a month's time infection is less likely to take place. The result of field experiments during the past summer indicates, however, that fields of several years standing may, under some circumstances, be benefited by artificial inoculation, either by (1) using the liquid culture as a spray, or (2) mixing with a quantity of soil to use as a top dressing.

H. W. Dunlap, of Holland Patent, N. Y., having more of the liquid culture than could be used for some seed he was inoculating, mixed it with a light loam and spread it upon a part of a field already in

clover. "The difference in color and size of the plants later on indicated perfectly where the soil had been distributed."

Mrs. J. A. Wells, of Bryn Athyn, Pa., tricd watering pea vines a month old with culture liquid, and "the treated vines were fully twice the size of the others."

A report upon a four-year-old alfalfa field to which bacteria were added, made by U. J. Hess, of North Yakima, Wash., reads as follows:



Fig. 6.—Roots of hairy vetch showing nodules.

The crop, which had been short, pale, and spindling, took on a darker color and a rank growth, and yielded, I think, about three times as much as formerly.

F. G. Short, of Fort Atkinson, Wis., writes:

In July the Department sent me a sample of alfalfa bacteria, with directions for application. This was used on a field of alfalfa which has been newly seeded this spring and up to that time had shown a very small growth of yellow, rather stunted plants. I used the bacteria according to directions and can see there is quite a decided change for the better.

In this case the field was gone over with a sprinkling cart containing a large quantity of the liquid cul-

ture. By attaching to the sprinkler an inch pipe 16 feet long, drilled full of small holes, a sweep one rod wide was made, so that the application was comparatively inexpensive.

In the light of these and similar experiments, there can be no doubt that bacteria of a high state of virulence are capable of producing inoculation at practically any time during the life of the legume if the conditions in the soil arc favorable. It is probable that similar results have not been previously noted, because bacteria of such high efficiency have not been used. While it can not be stated that as satis-

factory an inoculation will be obtained in this way as by treating at the time of planting, it certainly seems that under most circumstances where a crop is failing for the lack of nitrogen-fixing bacteria it is worth while making an effort to introduce them, even though the plants be several years old.

### PREPARING LIQUID CULTURE FOR FIELD USE.

While no special knowledge is required in preparing a liquid culture from the dry culture used as a "starter," it is distinctly necessary that

the user be possessed of plain common sense, the ability to read understandingly, and willingness to comply with directions. If the housewife mixes yeast in the sponge and leaves the pan in a chilly part of the room she is not surprised when she finds in the morning that the yeast has not "worked." If the dry culture is treated with a similar disregard for explicit instructions and ordinary prudence, the culture must not be held to account if the water fails to become cloudy. It would seem impossible, from the plainness of directions given (see p. 20), to make such mistakes as were revealed in a few reports. Using a "pickle pail" where a clean bucket is required, boiling or placing the culture on ice, and putting unopened packages in a hole in the ground afford examples of such blunders. Cultures plainly marked for "red clover," "common peas," etc., have even been used to treat such nonleguminous plants as corn,



Fig. 7.—Roots of velvet bean showing nodules.

tomatoes, lawn grass, and house plants. As the principles underlying soil inoculation become better understood, failures due to such mistakes will be eliminated. Notions of magical power will be replaced by the recognition of logical processes.

#### APPLYING THE LIQUID CULTURE.

After the water containing the nutrient salts and bacteria-laden cotton has been allowed to stand until it becomes milky, it is necessary to introduce this culture into the ground. This may be accomplished in

two ways—either (1) by moistening the seeds with the fluid, the bacteria adhering to their surfaces and consequently being in close proximity at the time of germination, or (2) by mixing earth or sand with the culture and spreading over the field as one would apply fertilizer. Greenhouse and small plat experiments indicated no particular advantage of one method over the other, and the hundreds of reports received from all over the country show that either means of introducing the organisms will produce satisfactory results if the directions are properly followed.

The sheet of directions which has accompanied each package of inoculating material, sent out by the Department of Agriculture, reads as follows:

#### Directions for Using Inoculating Material.

(Method patented in order to guarantee the privilege of use by the public. Letters patent No. 755,519 granted March 22, 1904.)

Put 1 gallon of clean water (preferably rain water) in a clean tub or bucket and add No. 1 of the inclosed package of salts. Stir occasionally until all is dissolved.

Carefully open package No. 2 and drop the inclosed cotton into the solution. Cover the tub with a paper to protect from dust, and set aside in a warm place for twenty-four hours. Do not heat the solution or you will kill the bacteria—it should never be warmer than blood heat.

After twenty-four hours add the contents of package No. 3. Within twenty hours more the solution will have a cloudy appearance and is ready for use.

To inoculate seed.—Take just enough of the solution to thoroughly moisten the seed. Stir thoroughly so that all the seeds are touched by the solution. Spread out the seeds in a shady place until they are perfectly dry, and plant just as you would untreated seed. If bad weather should prevent planting at once, the inoculated seed, if thoroughly dried, may be kept without deterioration for several weeks. The dry cultures as sent from the laboratory will keep for several months. Do not prepare the liquid culture more than two or three days previous to the time when the seeds are to be treated, as the solution once made up must usually be used at the end of forty-eight hours.

To inoculate soil.—Take enough dry earth so that the solution will merely moisten it. Mix thoroughly, so that all the particles of soil are moistened. Thoroughly mix this earth with four or five times as much, say half a wagonload. Spread this inoculated soil thinly and evenly over the prepared ground exactly as if spreading fertilizer. The inoculated soil should be harrowed in immediately.

Either of the above methods may be used, as may be most convenient.

In addition to the above directions an information card accompanies each culture, reading as follows:

# Information for Users of Inoculating Material.

The inclosed package marked "No. 2" contains a dried culture of bacteria. This culture treated according to the accompanying directions will produce a liquid culture, which, if associated with the proper plants, is capable of rendering available to these plants the free nitrogen of the air. This is accomplished through the formation of root nodules.

The bacteria are beneficial only in connection with legumes ("pod-forming" plants), and are not applicable to other farm or garden crops. Each culture is adapted to a

particular legume crop, the name of which is stamped upon the package. Even with legumes, these bacteria are of no decided benefit except when the proper nodule-forming bacteria are lacking in the soil, but a crop of nodule-bearing legumes improves the soil for succeeding crops.

The bacteria are capable of making up for a deficiency of soil nitrogen, but where other elements, as potash and phosphoric acid, are lacking, inoculation will not do away with the necessity for fertilizers containing these substances. Mineral fertilizers should, however, never be applied so as to come in direct contact with inoculated seed.

This material is furnished you with the understanding that you will carefully follow directions in its use, and will report upon the inclosed card your success or failure.

Methods of treating seed .- In the inoculation of small areas or small lots of seed the mechanical operations are so simple as to need no special comment. Where several hundred bushels of seed are to be treated, special provisions must be made for thoroughly drying the seed, so as to prevent damage through molding. Where enough floor space is available, a few hours are sufficient to dry the seeds if they are kept well stirred and a good circulation of air is main-

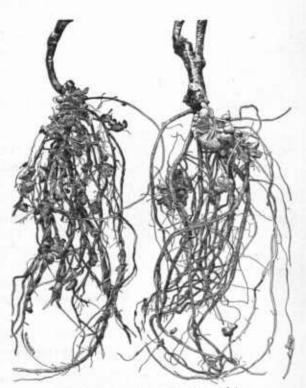


Fig. 8.—Roots of sweet pea showing nodules.

tained. On some ranches in the southwest carloads of seed have been successfully treated and dried in this way without injuring their germinative power. (See figs. 16 and 17.) The seeds are spread out and sprinkled with the liquid culture prepared in large tubs, care being taken to moisten every seed.

Another method, which permits no waste of the culture, is toplace the secd in gunny sacking, immerse in the fluid, allow them to drain, and then spread out to dry. In some cases special drying apparatus has been constructed, using a warm blast (never above 100°F.) and passing the moistened seeds along on a belt which deposits them thoroughly

dried in the saeks. This method lends itself well to the requirements of seedsmen who wish to treat several hundred bushels on short notice. "Inoculated seed" may soon be a regular item of trade. The fact that the bacteria thus dried upon the seed remain effective for inoculation purposes for several months makes this more than a possibility, and, with proper precautions, the effect upon germination need offer no objections. Indeed, evidence is not lacking that seed so treated—that is, with due regard to temperature in drying, etc.—is actually accel-



Fig. 9.—Roots of lupine showing nodules.

erated in its germination, an advantage which means much to the plant in a dry season and in the struggle against weeds.

R. C. Atkinson, of Wilkinsburg, Pa., reporting upon inoculated cowpeas and soy beans, has this to say: "Season was very unfavorable, yet the inoculated seed came to a nearly perfect stand and made a quicker growth. The difference was quite marked."

C. H. Elmore, of Applegate, Oreg., reports concerning alfalfa: "Seed all grew and lived through a dry summer on high, dry hill land; bids fair for a good crop next year. Without treatment the seed did not germinate at all."

W. H. Meyer, of Chatham, N. J., says of garden peas: "Seeds germinated somewhat sooner than those uninoculated. I recommend it to anyone to use in place of bone dust."

# USE OF FERTILIZERS IN CONNECTION WITH INOCULATION.

The object of inoculation is, of course, to enable the plants to secure an adequate supply of nitrogen without drawing upon

the soil resources. A deficiency of other necessary plant foods, such as potash and phosphorie acid, must still be taken into account, and where experience has demonstrated the need of these fertilizers no amount of inoculation can be expected to act as a substitute.

Some eare in the application of fertilizers should be observed in order to obtain the best results with legumes. Where soil tends to become acid and liming is not feasible, the use of eommercial preparations containing a high percentage of acid is of questionable advisability. Mr. C. R. Spencer, of Chardon, Ohio, has the following to say on this point: "For fifteen or twenty years we were very successful

in raising clover. At present we can not get much of a stand, and in many cases entirely fail. The complaint is general in this vicinity, some claiming that the acid used in phosphate is doing the mischief."

By using, wherever possible, fertilizers containing phosphates in a nonacidulated form (as ground phosphatic rock or bone meal) the tendency to create a soil reaction unfavorable to legumes would be largely avoided. Since the phosphoric acid in these fertilizers is in a form not at once available, the application should be made some time

previous to sowing, so as to permit the necessary decomposition.

Effect of fertilizers upon bacteria.-The effect of fertilizers upon the bacteria is also of some importance. The caustic action of concentrated fertilizers brought into close contact with inoculated seeds in sowing is known to be injurious. Especially is this true if the seed should still be in a moistened state after treating with liquid culture. Care should be taken that floors used for drying inoculated seed are thoroughly cleaned (brushed and scrubbed), especially



Fig. 10.—Roots of salnfoin showing nodules.

if the same floor space has served for mixing fertilizers. The liquid on the seeds would readily take into solution chemicals contained in the fertilizers, and the effect would be disastrous alike to seed and bacteria.

In no case should moistcned seed be dried by mixing with pulverized fertilizers, clean sand or dirt being in every way preferable if it seems desirable to hasten drying in this way. Indeed, it has been demonstrated in actual field experiments by Mr. M. B. Waite, of the Department of Agriculture, that there is a considerable injury to the seeds

themselves when sown in drills in contact with large amounts of mineral fertilizers used in full strength. Careful laboratory experiments a have established beyond a doubt the killing effect of commercial fertilizers upon seeds when in direct contact. If the germ of the seed can be thus injured when protected by a more or less thickened seed coat, the effect upon sensitive bacteria attached to the seed and unprotected can be nothing short of the absolute destruction of every organism touched. If the drill has previously been used with fertilizer, care should be taken to clean thoroughly all parts that may come in contact with the seed. If fertilizer is to be used, it should be spread and mixed with the soil or drilled previous to sowing inoculated seed.



Fig. 11.—Roots of garden pea showing nodules.

## CHOICE OF LEGUME FOR GREEN MANURING.

In selecting a crop to sow for bringing up worn-out or naturally poor soil, attention should be paid to its suitability for the soil, climate, and any peculiar local conditions. For instance, a plant requiring considerable warmth and sunlight, as the cowpea, should not be used as a cover crop in an old orchard affording much shade. For this purpose Canada field peas or clover would do better. In soils lacking lime (where lime is not easily obtainable) a plant capable of withstanding considerable acidity, as the cowpea or lupine, should be chosen rather than clover or alfalfa. In heavy clay soils holding much moisture, red clover or alsike clover will often succeed, though alfalfa can not possibly make a stand.

a Bul. 24, Division of Botany, U. S. Dept. of Agriculture.

Soil conditions vary so greatly even in limited areas that no hard and fast rules can be made for general application. Valuable hints

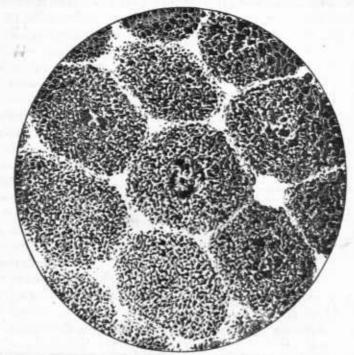
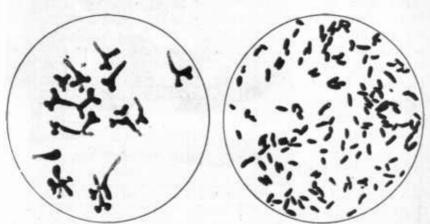


Fig. 12.—A few cells from a lupine nodule, magnified 1,000 times to show the bacteria.

may often be secured by studying the native or wild legumes, and it is too frequently the case that plants valuable as fertilizers are rated



clover nodule; magnified 2,000 times.

Fig. 13.—Branching forms of bacteria from a Fig. 14.—Rod forms of bacteria from a fenugreek nodule; magnified 2,000 times.

as mere weeds, and their benefit is thus lost by neglect or intentional eradication. The volunteer growths of sweet clover (Melilotus), Japan clover (Lespedeza), bur clover, beggar weed, and various wild beans and peas do much to improve land in disuse; and where they will not interfere with subsequent planting they should be encouraged to take

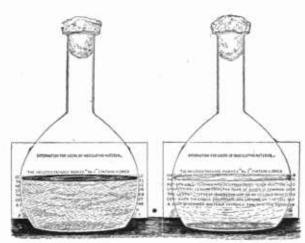


Fig. 15.—Clouding of culture liquid (in flask on left) due to the growth of bacteria in forty-eight hours.

possession to the exclusion of non-leguminous plants, especially those usually rated as weeds.

In this connection it would be well to urge the wider use of such legumes as crimson clover and hairy vetch, which are capable of making growth in a season of the year when land is usually (or frequently) not

bearing crops. Their effect in binding soil, preventing loss by washing, choking out weeds, etc., during the fall, winter, and early spring months is a consideration only secondary to their great ferti-

lizing value when associated with the nitrogen-fixing bacteria.

It is not possible in a publication of this kind to take up in detail the adaptability of the various legumes under diferent conditions, but the farmer who has difficulty in selecting a suitable soil-renovating crop need only to write to his State experiment station or to



Fig. 16.—Sprinkling Canada field pea seed with culture liquid; method employed on a large fruit farm in California.

the United States Department of Agriculture to receive publications or suggestions to guide him in a wise choice.

a Farmers' Bulletin No. 16, U. S. Dept. of Agriculture, Leguminous Plants for Green Manuring and for Feeding.

# WHEN SHOULD THE FARMER RESORT TO ARTIFICIAL INOCU-LATION?

The question naturally occurs to the farmer who reads of the wonderful work done by bacterial cultures, "How can I benefit by this discovery?" To begin with, it should be clearly understood that the nodule bacteria are only useful with the plants which Nature has adapted to pro-

duce root nodules when these bacteria are present. These plantsarepractically all included in the leguminous or "pulse" family, and the common forms sown are the clovers. peas, beans, and vetches. Some experiments have been made by workers in this country to infect the roots of corn, wheat, etc., with nitrogen-fixing bacteria, but the results were negative in every case.



Fig. 17.—Stirring seed moistened with culture liquid to hasten drying.

Assuming that the farmer has decided to sow some legume—alfalfa, for instance—the first question is, "Does my land need this treatment?" The answer depends on several conditions.

#### WHEN INOCULATION IS NECESSARY.

(A) Inoculation is absolutely necessary when-

(1) The land is at all poor or "thin" and has borne no legumes

previously.

(2) The land has borne legumes whose roots were devoid of nodules. Even in soils rated as "standard," and capable of producing excellent crops of grain, etc., legumes lacking nodules frequently make a very poor showing.

#### WHEN INOCULATION IS DESIRABLE.

(B) Inoculation is highly desirable when-

(1) The legumes previously grown on the land belong to another group of these plants. For instance, Bokhara or sweet clover (Melilotus) and bur clover are the only common legumes which give evidence of having nodule bacteria capable of infecting alfalfa. Infection of soy beans grown in the United States was first secured only after using soil imported from Japan.

(2) The soil produces a sickly growth of legumes even though their roots bear nodules. This applies whether the leguminous crop to be sown is the same as the preceding one or not. The introduction of the more active organisms furnished by pure

cultures may solve the difficulty.

#### WHEN INOCULATION IS WORTHY OF TRIAL.

(C) Inoculation is worth a trial when-

(1) The crop, already sown, has made a stand but gives evidence

of failing from lack of the nodule-forming bacteria.

(2) A field, which has previously grown a good leguminous crop, begins to give even a slight indication that, all other conditions being the same, it is not producing the highest yield. This situation is the hardest to detect, because it depends upon a gradual loss of virulence of the bacteria already in the soil, and the only way of being certain of this condition is to try inoculation and note results.

#### WHEN INOCULATION IS UNNECESSARY.

Inoculation is unnecessary—

- (1) In soils where the leguminous crops usually grown are producing up to the average and the roots show nodules in normal numbers. In such cases inoculation will give no material increase in yield nor will the soil receive additional enrichment thereby. This may be accepted as a general rule, although cases have been reported showing increases beyond what could be reasonably expected, due, in all probability, to the greater activity of the bacteria grown in pure cultures. Whether the increase under such circumstances would be sufficient to make inoculation worth while would depend upon the degree of deterioration which the organisms already in the soil had reached.
- (2) In soils rich in nitrogen. Where plants can secure combined nitrogen in the soil they will draw from this direct source even though they are provided with root nodules. Mention has already been made of the effect of rich soil upon the ability of the bacteria to

form nodules. Growing legumes upon such soils where nodules are not readily formed is not advisable, as it is manifestly poor economy. With the aid of the nodule bacteria, legumes can be made to produce quite as well upon much poorer soils. Ground containing a high percentage of available nitrogen would thus be released for the growing of grass, grain, or truck crops which do not possess facilities for utilizing atmospheric nitrogen. Of course, the use of rich soil would be justified if the legume crop should happen to be the most profitable in the region, or if the land should be of uniform fertility.

#### WHEN TO EXPECT FAILURE WITH INOCULATION.

Failure with inoculation may be expected-

(1) When the directions for preparing the liquid culture are not carefully followed. Reference has already been made to the necessity for strict adherence to a few simple but necessary instructions. In one instance, two dry cultures derived from the same "stock culture" and used by the same experimenter gave widely different results in adjoining fields. The first, prepared without proper reference to temperature and manner of application, resulted in the loss of the seed sown (alfalfa), no nodules being formed. In the second experiment, with more care taken, nodules were produced in abundance and the stand was a perfect success. The culture does not itself contain the nitrogen, but simply the organisms which potentially have the power of fixing nitrogen, and which, if properly handled, will increase in such numbers as to be of material benefit to the plants with which they become associated.

(2) When the ground is already thoroughly inoculated.

(3) When the soil is so rich in nitrogen as to prevent the growth of nodule-forming bacteria.

(4) When the soil is too acid or too alkaline to permit the development of either plants or heatering

ment of either plants or bacteria.

(5) When the soil is deficient in necessary plant foods, such as potash and phosphoric acid, as well as in nitrogen.

It should also be borne in mind that inoculation will not overcome poor results due to bad seed, improper preparation and cultivation of

the land, and decidedly adverse climatic conditions.

Before attempting to secure the benefits of inoculation, the farmer should first thoroughly inform himself upon the general culture of the crop to be sown. Neglect to do this simply invites failure. Sowing alfalfa on hastily prepared land, on land foul with weeds, etc., has been responsible for several hundred failures among our own experimenters, and through the country at large the percentage is certainly fully as great. The readiness with which free publications covering the essential points in the culture of all common legumes may be

obtained from the State experiment stations and from the United States Department of Agriculture renders it inexcusable to fail through ignorance.

#### CIRCULAR OF INFORMATION TO FARMERS.

The following circular letter was prepared as a reply to applicants desiring to gain some experience with the pure-culture method of inoculation:

Dear Sir: Your recent letter relative to nitrogen-fixing organisms has been received.

The organisms for the common legumes, such as alfalfa, peas, beans, clovers, vetches, etc., will be distributed to applicants who desire to aid in testing the efficiency of these organisms in different parts of the United States. As a general rule the quantity sent to each applicant will be sufficient to inoculate about two bushels of the seed for which the inoculation is desired. In special cases, however, when large quantities of seed are to be inoculated, directions for preparing the culture liquid will be forwarded, and as much as is desired can be made up at a cost of a few cents per gallon.

If you wish to secure inoculating material, it will be necessary to write us again, stating what legume you expect to sow and giving approximately the date of planting, so that we may send you the organisms in the best possible condition. This is necessary, as our methods require the inoculation to be made either before or at the time of planting the seed. Full directions for use are included in each package sent out.

The bacteria are beneficial only in connection with legumes ("pod-forming" plants), and are not applicable to other farm or garden crops. Even with legumes these bacteria are of no decided benefit except when the proper nodule-forming organisms are lacking in the soil, but a crop of legumes with nodule-forming bacteria improves the soil for succeeding crops. The inoculation process does not interfere with the usual method of sowing or subsequent cultivation.

When applying for inoculating material do not neglect to state the probable time of planting, kind of seed, and amount to be treated. In replying, please refer to this circular.

As the supply of inoculating material is limited, you should furnish the necessary information at once. Do not apply for these organisms unless you are reasonably sure your soil needs inoculation.

Yours very truly,

A. F. Woods, Pathologist and Physiologist.

Approved:

B. T. Galloway, Chief of Bureau.

The interest which this form of soil renovation has aroused may be indicated by the fact that for spring sowing in 1905 the number of applicants that could possibly be furnished with these experimental outfits (in all, 10,000) was listed by February 15.

It should be added that no formula or recipe will enable a farmer to produce his own cultures. The pure culture, used as a "starter," can be prepared only by a trained bacteriologist with laboratory facilities. In the "special cases" referred to in the above circular letter information is merely given as to what chemicals to buy in preparing large amounts of liquid culture from the dry culture furnished.

#### FIELD TESTS BY PRACTICAL FARMERS.

All of the foregoing discussion regarding the benefits to be derived from inoculation and the methods devised for propagating and distributing the nitrogen-fixing bacteria amounts to nothing unless it can be shown that these cultures really accomplish, under the general conditions to be found upon any farm, a decided increase in a crop over one grown without inoculation. In order that the bacteria might have the most thorough practical test possible the Department of Agriculture conducted during the year 1904 one of the largest experiments of this nature ever undertaken by any country. By the distribution of cultures to practically everyone who was sufficiently interested to request a package it was possible to secure about 12,500 tests under the most varied conditions in almost every State of the Union.

#### DISTRIBUTION OF INOCULATING MATERIAL.

The following list indicates the number of packages distributed in each State up to November 1, 1904:

Table I.—Number of packages of inoculating material (or inoculated seed) distributed from November, 1902, to November, 1904, arranged by States, Territories, and foreign countries.

		Clover.		Pea.			Bean.				ons.	
Where sent.	Alfalfa.	Red.	Crimson,	Garden.	Cowpea.	Field.	Common.	Soy.	Velvet.	Vetch.	Miseellaneous.	Total.
State or Territory: Alabama Alaska Arizona Arkansas California Colorado Connecticut Delaware District of Columbia Florida Georgia Hawaii Idaho Illinois Indiana Indian Territory Iowa Kansas Kentucky Louisiana Maine Maryland Massachusetts Michigan Minnesota Mississippi	242 11 64 171 20 30 10 28 40 16 147 239 4 16 147 239 10 91 11 12 66 50 50 10 91 11 11 12 10 10 10 10 10 10 10 10 10 10	17 1 15 21 3 9 2 12 12 17 17 101 103 4 4 41 132 556 3 21 28 28 29 29 29 20 20 20 20 20 20 20 20 20 20 20 20 20	10 6 4 226 3 3 5 	10 1 9 61 8 8 8 2 6 6 92 2 13 1 1 7 7 7 13 6 6 9 8 71 220 6 2	28 15 3 1 2 28 45 2 2 39 31 6 13 22 14	1 120 1 1 5 2 3 2 1 1 1 1 4 5	77	10 2 3 3 4 4 2 1 1 33 16 9 10 9	8 1 2 221 3 2 2 1 1 1 1 1 2 1	1099 2 300 1 1 1 2 2 6 6 177 45 5 4 8 8 6 6 8 8 8 4 4 3 3 20 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	79 1 10 23 2 4 4 3 4 4 3 4 48 36 32 11 31 25 5 9 9 10 26 5 19	608 2 14 14 137 490 47 63 33 77 184 287 164 201 102 209 218 104 61 174 305 302 95

Table I.—Number of packages of inoculating material (or inoculated seed) distributed from November, 1902, to November, 1904, etc.—Continued.

		Clover.			Pea.			Bean.			ous,	
Where sent.	Alfalfa.	Red,	Crimson,	Garden.	Cowpea.	Field,	Common,	Soy.	Velvet,	Vetch.	Miscellaneous,	Total.
State or Territory—Cont'd.  Missouri Montana Nebraska Nevada New Hampshire New Jersey New Mexico New York North Carolina North Dakota Ohio Oklahoma Oregon Pennsylvania Philippine Islands Porto Rico Rhode Island South Carolina South Carolina South Bakota Tennessee Texas Utah Vermont Virginia Washington West Virginia Wisconsin Wyoming	2111 344 966 3 111 688 188 285 2000 225 273 722 126 128 128 129 276 276 276 123 541 106 34 511	63 10 10 19 22 2 2 174 54 16 157 2 2 99 108 2 5 5 5 10 21 184 81 11 19 45	122 199 64 9 22 3 15 3 48 7 7 3 3	100 1122 7338844 420 11233888 517713322 663262627 553131	20 4 1 2 7 25 48 23 12 2 14 3 28 1 28 28 1 1 14 3 3 3 3 8	18 3 2 2 2 6 3 1 1 6	8 14 22 777 5 4 24 24 2 15 5 3 100 7 7 2 2 8 8 7 10 1 1	13 3 2 2 2 2 300 111 25 4 4 3 17 17 1 3 1 1 2 2 5 5 2 2 48 8 3 6 6 11 1	1 5 2 1 1 2 2 1 5 5 1 1	77 44 22 5 21 67 1 100 1 61 13 35 1 9 3 3 566 22 2 7	29 4 6 1 1 8 8 3 3 4 4 4 7 7 2 2 2 2 9 7 7 7 2 8 6 3 3 3 7 7	367 50 137 6 68 156 24 24 24 24 24 11 18 26 24 11 137 27 137 27 24 11 137 27 27 27 27 27 27 27 27 27 27 27 27 27
Foreign countries: Australia British Guiana Canada Costa Rica Cuba England France India Mexico South Africa Unclassified	2 1 6 2 3 2 2 3 1 378	6 1 3 1 146	1 1 79	1 2 2 3 2	1 5 3 1 370	1	1 1 3 3	2 1 1 2 2 54	3	1 1 2 2 1 24	1 1 3	7 1 18 5 17 10 6 13 4 8 1, 287

#### REPORTS SHOWING PROPORTION OF SUCCESSES AND FAILURES.

While it has been impossible to obtain reports from all experimenters the percentage of replies has been unusually large, and is quite sufficient to enable the formation of a fair opinion as to the value of the cultures distributed. In calling for a report it was, of course, understood that in some cases where the culture was used the resulting crop could not be a success, and the users were asked to indicate, as far as possible, when the failure was evidently due to some fault of seed or weather. Likewise, if the soil was shown to have been stocked previously with the proper bacteria and good crops were produced, the use of inoculating material was not expected to be of benefit, and no difference would be detected upon treated and untreated land. It is obviously difficult, however, to get all experimenters to make a note of these conditions, a report upon the general result being about

all that can be expected in most cases. For this reason the summary of the reports is not as favorable for inoculation as it probably would be if all of the experiments could have been followed in the same way as is possible when such investigations are conducted upon a small scale. It should also be remembered that no selection of the region in which tests were to be made was possible. Experiments with inoculated seed of crops manifestly unadapted for the locality in which they were sown which were reported as a failure of the inoculating material have been recorded as such. In spite of counting unfavorable reports of this kind, which by no fair adjustment should be included, but which, on account of the impossibility of being certain of the conditions, could not be thrown out, the average percentage of failures is less than is generally expected from the indiscriminate planting of seed known to be good.

The tabulated reports so far as received up to December 31, 1904, for all of the principal crops are as follows:

Table II.—Reports of inoculation experiments with principal crops.

	1.	2.	3.	4.	5.	6.
Crop plant.	Total num- ber of reports.	Inocula- tion result- ing in definite increase of crop.	Failures definitely ascribed to bad season, poor seed, weeds, etc.	in crop; organisms already	No evident advantage from inoc- ulation; nodules not formed.	Percentage of successful experiments.
Alfalfa Red clover	1,380 680	667 391	399 156	70 98	244 35	73 92
Garden pea Common bean	276 305	144 139	64 82	46 49	22 35	87 80
Cowpea	492	253	95	99	45	85
ouy bean	175	61	43 19	12 10	59 13	51 75
Hairy vetch.	80 67	36	21	5	5	88
Callada neld nes	23	23	7	7		100
v civel bean	11	11	6	5	6	65
AINIRC	8 10	3	1 3	3 2	1	75 100
weet pea Berseem	2	1			1	50
Total	3,540	1,772	896	406	466	679

aln computing the percentage of successful trials the number of cases where there resulted no evident advantage from inoculation (fifth column) has been compared with the number which were Positive successes (second column), experiments under columns 3 and 4 being, of course, disregarded.
b Average.

#### SOME CHARACTERISTIC EXPERIENCES.

It is believed that a careful examination of replies received from experimenters who have had no other instructions than those sent with the inoculating material will demonstrate the success of the new methods devised by the Department of Agriculture in a way that would be impossible by a mere discussion of results obtained in the laboratory or greenhouse. Such intense interest has been shown throughout the whole country in succeeding with alfalfa that, for this crop, reports have been selected showing the distribution and results

by States. The reports representing the various States are in many cases far from being the best received, selections being made to show the possibilities of soil inoculation under widely different climatic and soil conditions. For the other crops, only such are given as illustrate some special feature or furnish suggestions to prospective experimenters.

#### Alfalfa.

- Alabama, Tuscaloosa. T. J. Ozment.—Seed treated with the bacteria produced 100 per cent more than untreated seed.
- Arkansas, Mount Ida. D. Peters.—Made a crop where it would not grow before.

  Am of opinion that the inoculation is all right.
- California, Sanger. E. C. Southworth.—I had only material enough to inoculate 5 acres; seeded 25 acres to alfalfa. The inoculated seed grew, the other did not.
- COLORADO, Flagler. Edwin Forrest.—I think it was greatly improved, as I got a stand which I never accomplished before. It grew to a height of 8 to 12 inches, bloomed, and made some seed. I left the crop standing as a mulch over winter. I planted in rows 16 inches apart so I could cultivate with a sugar-beet cultivator.
- Connecticut, Granby. Daniel P. Cooley.—The nodule formation is perfect. No crop has been harvested this season. Has been cut four times to kill weeds. The stand at this date (October 20, 1904) is good.
- Delaware, Townsend. James Flanagan.—A heavy storm just as plants came up covered many of them, but those remaining looked nicely and have bacteria nodules on the roots.
- IDAHO, Freese. I. E. Lobaugh.—Made fine growth for first year. Good stand. Clipped three times. Left on ground.
- ILLINOIS, Mount Carmel.—W. F. Chipman.—Nodule formation on almost every plant examined yesterday, and foliage rich dark green. Fine, vigorous stand. I find more nodules on the alfalfa sown about a month ago than on that sown last May. The reason perhaps is the difference in the amount of ground covered with each package, the first being about 9 acres, while the last was spread over but 1 acre.
- Indiana, Butler. L. G. Higley.—Bacteria for alfalfa was received in good condition about May 1. I prepared it and mixed it with about 20 bushels of rich soil and sowed it on the field after plowing it. I sowed my alfalfa seed May 11, along with 2 bushels of smooth barley per acre. It has done better than any alfalfa that I ever sowed. It stands over a foot high nearly all over the field. There is hardly a square foot of land in the field that is not well set with plants. I took a spade to-day and went in the field to see if I could find any trace of the bacteria, and I soon found that the soil was full of it, every plant having lots of the nodules on the roots. I then went to a field of 2-year-old alfalfa, which never was treated with bacteria, to see if there were any nodules there, and after hunting a long time I found a few very small nodules, but hardly enough to be really worth mentioning. This field is failing and I will have to plow it up. Alfalfa will grow on real rich soil without its bacteria, but I believe it will grow better with it; and if the land is the least bit poor it will starve to death if it has not its bacteria.
- Indian Territory, Pecasset. Don Nolian.—We had tried it (alfalfa) twice before, this being the third trial. Had perfect stand and have cut three crops and have good covering for winter. There is about 1 acre in lot, and I have taken about 3 tons of hay from it, or a ton at each cutting.

Iowa, Algona. Judge W. B. Quarton.—I took one gallon of nice rain water and followed the directions received from your Department with the culture of bacteria, and received the identical results that your Department said I would. I personally inoculated this bushel of seed, then spread it out to dry, took it to the farm the next morning, and planted the seed. \* \* \* I have been upon my farm many times between the middle of July and this writing, taking my pocket knife and digging down to the roots of the alfalfa plant. I have never failed to find plenty of thrifty-looking tubercles on the roots, they ranging from one to clusters of one hundred, and I am satisfied that my field is thoroughly and completely inoculated, and I believe that your method is a complete success. \* \* \* I feel like congratulating your Department upon the very thorough and practical work that you are doing in the line of plant industry and especially as to leguminous plants. I hope that you will continue it, because the legume is the one plant above all others that fertilizes the soil and at the same time furnishes the protein necessary to balance the food ration in our corn-growing States like Iowa.

Kansas, Halstead. A. Murray.—The alfalfa inoculated could not have done better.

I will not plant any after this without inoculation. I think inoculated alfalfa

is as good at 1 year old as uninoculated is at 3 years old.

Kentucky, Berlin. John A. Buser.—One acre was planted; one-half was inoculated, the other half was not. Received good stand in all parts. On examination of some roots the treated plants had root nodules and the untreated were barren.

MAINE, Wayne. S. H. J. Berry.—Last year I tried to raise alfalfa but was unable to get a stand, but this year, by the use of the inoculation, I have a very pretty plot of this valuable grass. I believe it to be what my land requires.

Maryland, McDaniel. William Bielefeldt.—Inclosed please find your card filled out as to general results. I did not harvest any hay off the field, but pastured it lately. I am sorry that I am not able to give you any definite figures on the crop, and as your card is not large enough to express my appreciation and enthusiasın for your method of inoculation you will please excuse this letter, in which I will try to sum up my observations of the experiment in the following: I inoculated 1,800 pounds of alfalfa seed with the material received. I dried the seed well after inoculating and sowed it from May 1 to August 15. The land is a medium heavy fine clay soil and originally, I think, a fairly good soil, but has been entirely farmed to death with continuous tobacco raising, and after that wouldn't grow any more they followed it up with wheat and corn till that failed to grow any more; then the farm was sold. So I can say the soil is in a very poor condition chemically and physically, so much so that on 2 acres sown with seed not inoculated, alfalfa failed to make a stand at all. But on all ground in the same condition the inoculated seed made a brilliant stand and is looking a real deep green in color, when nearly everything else is dried up, as we have had no rain for six or seven weeks. In all, allow me to say that in my opinion your Burcau has made the greatest discovery toward helping the growing of alfalfa that could be made, and that you may well be proud of it, and I thank you for giving me a chance to use it. A neighbor adjoining me sowed uninoculated seed three successive times on the same piece of ground and failed to get a stand; that is positive proof of the inoculation being a benefit.

Michigan, Kiffie. A. L. Rockwell.—Mixed bacteria with soil; sowed broadcast after seeding; harrowed lightly. Seed all grew and made a good stand. Other

seed without bacteria failed.

- Missouri, Levy. Thomas O. Hudson.—Planted in 1901. Alfalfa was sickly and yellow and spindling, and did not do any good till this year after inoculation. This year it has been dark green and thrifty, and I think it will be better next year.
- Nebraska, Atkinson. H. E. Henderson.—I got a good stand where I had failed twice before. I think it the only safe and sure way to secure a stand.
- New Jersey, Vineland. E. L. Bolles.—First cutting on May 25, 1904, of 2 to 3 tons from 1 acre (seeded August 25, 1903), nine months from seeding. Scores of trials without inoculation have been made in this section with universal failure. Alfalfa wintered well, while we had a killing winter for crimson clover.
- New Mexico, Nogal. Ed. C. Pfingsten.—Soil inoculated shows bacteria on all roots examined. Inoculated plants from 20 to 30 inches high, others 6 inches.
- New York, Amsterdam. Barlow W. Dunlap.—I have recently examined the plants in all parts of the field and find nodules on nearly every root. The same piece was sown with untreated alfalfa seed in 1902. The plants started well, but nearly all died before fall. I could not then find a single nodule on the roots.
- NORTH CAROLINA, West Raleigh. C. K. McClelland.—Four cuttings have been made; second and third cuttings contained much alfalfa. Examination shows plenty of tubercles on the roots, so inoculation was successful.
- Ohio Granville. Chas. B. White.—Excellent set. Contiguous soil, not inoculated, gave feeble stand. The ground was cut thrice, but left on field as mulch.
- Oklahoma, Lambert. T. W. Croxton.—Good, a perfect stand, and of healthy color.
  On upland prairie.
- Oregon, Days Creek. C. N. Wood.—My neighbor sowed alfalfa and red clover the same time I did, also with irrigation, equally as good seed, and equally as good or better soil, and his crop did not get large enough to clip at all this year yet, and it looks sickly, while mine is thick and a rich green in color. My crop of alfalfa and red clover is at least 60 per cent ahead of my neighbor's. Mine was inoculated and his was not. I shall use soil from the inoculated field to inoculate other fields of the same kind of crop.
- Pennsylvania, Muddycreek Forks. Vallie Hawkins.—Sowed 3 acres without inoculation last year. Good stand, but few nodules. Had to resow this year (August 2), and inoculated seed. Roots are well supplied with nodules and I have a good stand, 8 to 12 inches high, on October 18.
- South Carolina, Williamston. A. W. Attaway.—Very dry time on it, nevertheless a very good stand. Think inoculation very profitable. Others tried without inoculation and fell behind me.
- TENNESSEE, Columbia. Horace B. Hanson.—It has tubercles formed on the roots; is looking fine and healthy. Some of it is on very thin land. I have been trying this plant on the same land for three years without success.
- Texas, San Antonio. B. G. Barnes.—The inoculated appears to be more vigorous and healthy than that without inoculation, although the latter was planted first and originally came to a better stand by reason of the ground being in good condition at the time of planting, while the inoculated was not.
- Vernont, Randolph. John W. Burt.—We think the result is very good. If we had cut as a crop this season we would have gotten a good yield, and we are confident that next year will show satisfactory results.
- Virginia, East Leake. A. K. Leake.—It is 18 inches high and could not be more promising; looks splendidly. You will see by the samples I send you that it is full of nodules, showing in an astonishing manner the bacteria-bearing nodules. There are nodules on every plant I dug up. When I dug up some old plants from a field which has failed I saw no nodules. No one has ever succeeded with alfalfa here.

- Washington, Belma. Chas. Richey.—Inoculation very beneficial. Growth had formerly been very poor; plants turned yellow and many died, making it hard to get a good stand. Now difficulty is overcome.
- West Virginia, Berea. John E. Meredith.—Have been trying to grow alfalfa twelve years, and have now the finest prospect of success I have yet experienced.
- Wisconsin, Fort Atkinson. "Hoard's Dairyman," November 11, 1904.—An experimental trial of this method of inoculation was made by Professor Short, one of the editors of this paper, last summer with very evident success. Our field already shows the good effect of inoculation. (The method consisted in going over an alfalfa field which was not thriving with a sprinkling cart containing the culture liquid. The operation was comparatively inexpensive, as a 16-foot pipe drilled full of holes was attached to the rear of the sprinkling cart, the water thus taking a sweep of nearly a rod in width.)

### Red Clover.

- California, Arcata. William W. Turner.—A part of the ground was a loose sand, a deposit from the river. It was a hard matter to get anything to grow on it. Here is where my inoculated clover seed seems to grow and flourish. The rest of the ground was a sediment loam and very rich. It was not long before the pigweed started, and it came so thick that it choked out the clover, except what was on the sand. That is growing nicely; has a nice dark-green color.
- CONNECTICUT, Wolcott. Samuel Wilson.—I sowed about 8 pounds of seed, not inoculated, all over field and 3 pounds of inoculated seed in the form of a cross.

  Result, cross distinct with clover; balance of field noue.
- Illinois, Anna. J. W. Fuller.—Got good crop where I had failed eight years in succession.
  - Hillsboro. Thos. S. Evans.—I am pleased to report a complete success with the inoculated clover. The clover was the finest in this section.
  - Tumaroa. W. J. Appel.—Find that where I had failed to get a stand at previous sowing got fairly good one this time, and nodules of fairly good size on roots.
- Indiana, Colfax. T. C. Holloway.—The clover was pastured after the crop of wheat was taken off. Can give no exact figures. It was sown on white-clay land that has been producing very poorly. It now seems equally as good as that on the black land.
- Iowa, Muscatine. Charles A. Price.—The clover was sowed with oats. The oats showed an increase of 15 bushels per acre over oats on same ground where no treatment was given. An examination of the clover roots showed 75 per cent more nodule formation than on that from untreated seed.
- Kansas, Burlington. John W. Alexander.—Plots 1 and 2, 4 acres each, yielded threefourths to 1 ton per acre; seeded April 15. Plots 3 and 4, 1 acre each, not inoculated, but seeded at same rate and same time, very weak; not much growth and no hay cut.
- Kentucky, Adairville. Martin Boyd.—One of our farmers tried it last year on 10 acres out of 100. The 10 acres is all he has now standing.
  - Hopkinsville. Ben C. Moore.—I cut 2 acres of clover which had been inoculated and 2 which had not been, and find that there is a difference of about 500 pounds per acre in favor of inoculated seed.
  - Winchester. Dr. M. S. Browne.—I have 40 acres on which wheat and blue grass were sown in 1902, and clover in February, 1903. There was a severe drought in the fall of 1903 which killed the clover, leaving the stand of blue grass on which inoculated clover seed was sown in February, 1904. Clover sprang up in April and made an unprecedented growth. On this meadow were grazed from 200 to 400 sheep and 25 cattle to July 10, when all stock was removed. We cut a good yield of hay in the midst of drought, August 15. We never

before dared to graze spring-sown clover during the spring and summer, and very little in the fall. If we did and any drought came, it would die. This year the drought was worse and more protracted than last year, and the clover is healthy after all the grazing and mowing. This is unprecedented. Another 40 acres of similar land was plowed in October, 1903, and sown in winter wheat and blue grass. The wheat winter killed. This was sown with clover seed at the same time as the first field, but the seed was not inoculated. The result was a complete failure—the plants are mostly dead. This ground was prepared a year later and was in a good state to catch clover, but the inoculated field was really not in a fit condition when sown.

Maine, Augusta. John Jackman.—The very best results. I soaked or moistened seed carefully, as per directions, and reserved small piece of ground for test; rest of ground was sown to same kind of seed, but catch on inoculated patch is noticeably stronger. It seems as if every seed came up and grew.

MARYLAND, Grayton. Rev. William Brayshaw.—Report on clover sown September, 1903, at Valley Lee, Md. I sowed two lots of seed side by side, one inoculated, the other with 100 pounds of South Carolina rock. Inoculated made double the growth and bade fair to give three times the quantity of hay.

MICHIGAN, Fennville. Chas. E. Bassett.—Inoculated part of field gave 12 per cent more yield, and nodules on roots were as large as small peas, while on that not inoculated the nodules were extremely small.

MINNESOTA, Campbell. N. W. Ware.—Bacteria nodules show in abundance and plants very thrifty. Sown on 15 acres of northwest Minnesota Red River prairie soil with best results. Former owner tried in vain for years to get a stand of clover.

Missouri, Cabool. C. L. Morris.—Sowed two plots. Plot 1 was inoculated and has made a fine growth. Plot 2, not inoculated, has nearly all died out. Plot 1, a success; plot 2, a failure.

Hale. E. P. Swartz.—Mr. William Morris, following instructions, sowed 7 gallons of clover seed on 8 acres. The season was such that he could not suitably prepare the ground, simply sowing it on the hard ground. The third day he harrowed the sown piece of ground once; could not cross harrow it. I was over to see it this fall, and am pleased to report the most magificent stand of clover I ever saw. A neighbor whose lands adjoin Mr. Morris had carefully prepared his soil, sowed the same day, and he did not get one-half the stand that Mr. Morris obtained. I think you have made a most important discovery—one that is of incalculable benefit to the farmer.

Nebraska, Elgin. Marcus Brown.—Inoculation successful, nodules appearing quite plentiful, though uneven. Crop appears best I ever saw in Nebraska.

New York, Holland Patent. H. W. Dunlap.—My tenant reports the best stand he has had during his occupancy of the farm, and that upon a hillside where until then he had never been able to make red clover grow. Plants I examined in August showed nodules in every case. Having more of the culture liquid than could be used upon the seed, I distributed this on some light loam, which, after stirring and drying, was broadcasted upon a small part of a field already in clover. My tenant reports that the color and size of the clover indicated the distribution of the soil perfectly.

Poughkeepsie. B. W. Russell.—My soil is clay loam and I have always had trouble to retain clover through the first winter's frost. Have delayed in my report to see the action. Am very much pleased and can say it is retained thus far and the best prospect for a crop that I have ever had. I seeded 5 acres after oats and of course did not harvest a crop of clover last year. The oats were the best I ever raised. They were watched by my neighbors all the season and all have pronounced it a success.

- NORTH CAROLINA, Loftis. Benj. G. Estes.—I have a fine catch of clover where I have not been able to get clover at all. In fact, the farmers say clover will not grow here at all.
- Оню, Seaman. Ira C. Howard.—A fine set on clay upland. I sprinkled the water that was left after soaking the seed over the ground in spots; every spot is plainly, visible.

Pennsylvania, Southport. F. L. Bray.—Clover looks fine in lot where inoculation was used; scarcely any in lot where no inoculation was used. Both lots with same soil, same methods of cultivation, same nurse crops, and same time of sowing.

Virginia, Sandidges. W. S. Gill.—Seed inoculated produced clover 18 to 20 inches high at this time and blooming. That not inoculated 6 to 8 inches high and sickly looking; not blooming. I have all confidence in the "bug" and believe it will restore clover to us again.

Washington, Bothell. Harry G. Brower.—Mixed the material according to directions and thoroughly wet 10 pounds of red-clover seed three times and dried each time. What liquid 1 had left I mixed with 25 pounds of dry dirt and sowed this on 1 acre; harrowed three times. Season was very dry, but the seed lived through and the ground has a good stand. In fact, I am the only one who has a good stand. People told me the soil was too poor for anything.

West Virginia, Thomas. O. H. Hoffman.—The clover stand after the wheat was cut off last summer was the best that has ever been on the same farm for years. We had plenty of bacteria solution for this seed.

Wisconsin, Iron River. Joseph Yerden.—I had sowed clover on same land two years in succession and could not get a catch. I used the inoculating bacteria that you sent me and have a fine stand of clover.

# Cowpeas.

Alabama, Gateswood. Wm. C. Payne.—No apparent effect on old ground. On new good land, seed not inoculated did practically nothing and inoculated seed gave about 1½ tons per acre. A small plot of inoculated "Unknown" pea on good new land was immense. Yielded 3 tons per acre, and that not inoculated almost nothing on similar soil—new ground.

FLORIDA, Pensacola. Geo. W. Howes.—Result of inoculation good. I planted as a fertilizer on poor sandy black-jack land and got a third better results without manure, but inoculated, than on the same land with cotton-seed meal as a fertilizer.

Georgia, Bluffton. H. B. Harrison.-Increase of yield 100 per cent.

Rome. Hamilton Yancey.—The growth has been rank, of rich dark color over the entire field that was seeded. A difference in favor of the inoculated pea was quite noticeable. My neighbors and friends who have seen the field insist that the field is seeded with a different kind of pea. I wish to express to you my satisfaction and gratification with the experiment. I believe the work you are doing is of inestimable value to the farmers of our country in the future redemption and improvement of our lands.

Illinois, Mount Vernon. E. M. Dana.—Sowed in orchard. Each alternate space inoculated shows a great difference in rankness of growth over uninoculated,

especially on yellow soil badly worn.

Indiana, Milan. James Tribbey.—Cut for hay. Estimated difference between inoculated and uninoculated 300 per cent in amount of vines, hay, etc., in

favor of inoculated. No difference in amount of peas.

Kansas, Walnut. H. C. Coesten.—Inoculation was perfect and satisfactory. Would prefer this method of inoculation to the sowing of soil from field to field; by the latter a person is liable to transfer plant disease. I transplanted the leaf blight to my field a few years ago by doing so.

- KENTUCKY, Winchester. Dr. M. S. Browne.—Estimated weight of hay increased threefold or more; peas fully as much increased.
- LOUISIANA, Lafayette. Ray Fiero.—In 1903 I sowed peas on a side hill and the peas did not grow over 8 inches high, with very small nodules. This year the inoculated peas sown under same conditions made a growth at least four times as great.
- MARYLAND, Chaptico. William H. Gardiner.—The 2 acres inoculated grew twice as large, as peas were more prolific than uninoculated part. In fact, the 2 acres were the only part harvested. The rest of the field was insignificant.
- Missouri, Marionville. U. L. Coleman.—Where inoculation was used the peas did a great deal better and produced fully one-third more. I found few nodules where the inoculation was not used, but where inoculation was used the roots were literally hanging full of nodules, some as large as peas. I showed sam ples to several of our farmers, and they all stated they had never before seen as many nodules on one vine.
- NORTH CAROLINA, Asheville. Fred Kent.—Inoculation very good. Farmers in the neighborhood wish to know how such peas are grown, as theirs were failures.
- OKLAHOMA, McCloud. Jesse Hearn.—Rapid growth; quick development; 20 per cent increase in yield. Roots full of nodules. Land in fine shape for next crop.
- Pennsylvania, Hartstown. J. T. Campbell.—Where soil was inoculated the result was marvelous, four times as great as where there was no inoculation. Nodules one-half inch in diameter.
- South Carolina, Aiken. Miss Louise P. Ford.—On 1 acre we planted cowpeas broadcast. On one-half of this acre we planted one-half bushel of inoculated cowpeas, on the other half acre we planted one-half bushel of uninoculated cowpeas, plowing them both in just the same way. About the middle of June, when harvested, we gathered 1,375 pounds of hay from the inoculated half acre; from the uninoculated half acre we gathered 750 pounds. The land is known as poor sandy soil, and we did not enrich. This is the result of Miss Pellew's and my experiment on Twin Flower farm.
  - Orangeburg. F. M. Rast.—I tried the inoculated by side of stable compost and will say that it was just as good as those fertilized with compost. I am well pleased with results.
- TENNESSEE, Ripley. M. M. Lindsay.—Five times as much vines and leaves and two times as much peas as planted on same land without inoculation. There can be absolutely no doubt that above results are due to inoculating seed.
- Texas, Double Bayou. G. Wolff.—My oats, which I planted after inoculated cowpeas, look very fine and on land that is too poor to make a crop of cowpeas without inoculation. I am a strong believer in inoculating the seed.
- Virginia, Danville. T. L. Smith.—The pea vines were the finest I ever saw. I measured some vines 12 to 15 feet long. I made three times as much hay to the same quantity of seed as I ever made.
  - Ionia. R. Dewsbury.—Peas on same ground as last year were more than twice as good, and no help given. Last year had no nodules; this year had. Something increased the yield of peas and vines 100 per cent.

### Garden Peas.

- Figure A., Saint Petersburg. S. S. Stults.—Most excellent results, compared with what we usually get from same soil and same treatment. I got four times as many peas as I did without the microbes.
- MAINE, Lincoln Center. C. A. Brown.—Crop about double what I got with seed not inoculated. The stuff is worth a good deal for peas on my soil.

Massachusetts, Boston. Jesse M. Gore.—The pods were larger, fuller, sweeter, and two weeks earlier than peas planted at the same time and under similar conditions with the exception of the inoculation.

Michigan, Pellston. H. L. Millspaugh.—We planted four rows of each seed each way; that is, four using inoculation and four without it, harvesting the peas as a green table crop. The results were very flattering to the use of the inoculating material—fully double yield.

New Hampshire, Franconia. L. F. Noble.—There were bacteria bulbs everywhere more than an inch through. It was wonderful and it filled me with hope for

the future.

Pike. H. E. Howard.—Sown same day on ground side by side where bushes had been cut and burned, with no other fertilizer. Inoculated plot produced about three times as much as the other.

New York, Clay. Mrs. Arthur Hall.—Entirely successful. Yield wonderful. Culture applied to earth and sprinkled along pea rows. The soil now seems like sandy loam, whereas it was the heaviest of clay before. Celery following peas is very fine.

Northwood, John R. Spears.—The tall vine (3 feet high) was cut from a row that was treated with the culture of nitrogen-gathering germs. This sample fairly represents the growth of all the rows thus treated. The short vine (14 inches high) was cut from the row of vines not treated with the culture. It was the best vine among those untreated. The rows were 4 feet apart and the distance between the two plants was about 7 feet. If you recall that the seed was the Dwarf Alaska, the large vine will seem rather remarkable, I think. The nodules are particularly well worth observing. On July 3 I made the first picking from the plot. On 53 untreated vines, taken as they came, I found 102 pods; on 53 treated vines, taken as they came in the next row, I found 856 pods. The first picking well-nigh stripped the untreated row; the treated vines have yielded two good pickings since, and still another is now filling out. Vines first appeared above the ground on May 17, and they had reached a height of from 2 to 3 inches on June 1. The plot was then of uniform appearance as to the thrift of the vines. On June 1 I watered all the vines in the plot, except one row, with a solution or culture of those germs, made according to accompanying directions, and raked fine dry soil over the ground thus moistened. Since that date all the rows have been cultivated enough to keep the surface soil fine and free of weeds and grass, and all have been treated alike in every other particular. No fertilizer of any kind has been applied to any of the rows at any time before or since planting. The quality of the soil is uniform throughout the plot. The soil itself could have had no influence in producing the extraordinary difference in vine growth. If I seem to be burdening you with details, I must urge as an excuse the extraordinary interest excited by the wonderful success attained by the use of the nitrogen-fixing germs.

Utica. H. D. Pixley.—Every way satisfactory. Got as large a yield per acre as the five acres of peas in same field with heavy barnyard fertilization.

Pennsylvania, Bryn Athyn. Mrs. J. A. Wells.—On April 14 I planted three kinds of peas. They came up well, but did not grow rapidly. I had inoculated the seed according to directions. On May 14 a neighbor, having obtained a culture for peas, spared some for me. I inoculated more seed and planted them; then having some of the liquid left, I added water at the rate of one-half pint to 2 gallons of water, and having hoed the soil away a little from the roots of the previous planting of peas (now 4 or 5 inches high), I watered them with the diluted culture and hoed the soil back. Well, now the watered planting of peas is a sight—tall, luxuriant plants covered with fine pods. They are the

admiration of the neighborhood. The later planting that was inoculated but not watered with the culture is doing better than any peas I have had heretofore, but not nearly so well as the ones that were watered after they were up.

[Later report.] Four bushels of fine, well-filled pods were gathered. Hitherto our soil would not produce peas to amount to anything. My next-door neighbor has soil exactly similar to ours and manured it more heavily. He used the same seed as I did, but my peas were decidedly finer.

Philadelphia. S. N. Lowry.—Vines yielded once and a half the crop yielded by vines from ground not inoculated but which was manured. The vines from inoculated seed yielded full pods and the peas and beans were larger than

those from untreated seed.

Westchester. Edw. H. Jacob.—Inoculated peas fully matured by October 1; uninoculated did not flower at all. On September 15, 1904, inoculated peas were 18 inches high, uninoculated 8 inches high. Planting was late, but shows big returns by inoculation. (Date of planting, August 15.)

South Carolina, Gaffney. Jeremiah Gardner.—My peas were better than the peas of others who used commercial fertilizer; ripened early and evenly; circum-

stances unfavorable. I consider inoculation a boon to agriculture.

South Dakota, Lead. A. L. Read.—Sowed on yellow clay. Had great difficulty to loosen the ground enough to cover the seed. Impossible to cultivate. Harvested about 17 gallons of peas of well-filled pods. On piece of ground same size, seed not inoculated, harvested less than one-half gallon of peas.

Wisconsin, Janesville. J. T. Fitchett.—Plants were stronger, blossomed two weeks earlier, stood dry weather better, and matured more peas than plants not so treated. In addition, I inoculated seed for four other parties, requesting them to report to me. One man reports 50 per cent better yield. His soil was poor, and the bacteria showed more effectively by contrast. A market gardener reports a larger yield than from similar seed not treated; but to him the best feature was earlier maturity by two weeks. All report favorably, those planting on poor soil reporting the largest increase.

#### Beans.

Alabama, Fruitdale. George W. Dibble.—When the crop was ready for market the beans were picked from both plots. The plot that was inoculated kept growing and bearing fruit; on the other plot they dried up. When the beans were gathered, the yield on the inoculated plot was more than double that of the other.

COLORADO, Arvada. A. B. Cole.—Planted 3 acres adjoining 2 acres uninoculated.

The inoculated beans produced one-fifth more to the acre than adjoining.

Illinois, Chicago. Stuart S. Crippen.—Yield of beans was one-third above average, and product unexcelled in size and flavor for table use. Seed beans are con-

siderably larger than parent beans.

Massachuserrs, North Falmouth. Ella M. Donkin.—The beans were the admiration of all who saw them, and I invited all whom I could interest in them to see them. I had planted in another part of the same garden beans which, although supplied with fertilizer, did not amount to anything, and I decided to try the bacteria organisms, even if it were late in the season for planting. I planted them July 14, and early in September we had fine string beans to use. The pods were large and of excellent quality. They continued to bear until an early frost killed the vines. \* \* \* We examined the roots in different stages and found the nodules well developed.

Worcester. James T. Rood.—Result of inoculation very satisfactory. Inoculated bed and soaked beans. Tried both separately and together. About equally

good in results. Inoculated plants caught up with previously planted uninoculated plants and gave more and better yield. Foliage greener and healthier.

Michigan, Saugatuck. F. M. Kreusch.—I gathered the beans about September 20; have only thrashed part of them, but I am sure I will have five times as many as last year on the same ground. I think it is immense.

New York, Kingston. Mrs. Clara N. Reed.—Pods very full of large beans. Some vines had a second crop. The inoculation has greatly enriched the soil, so

that it is much better to use for other vegetables.

Penn Yan. John D. Buckley.—The ground was on a side hill, gravelly and sandy, and had been practically worked out. In spite of this and insect attacks I had the best piece of beans I ever raised. A farmer living near me planted beans twice in succession on the same land and I helped harvest the beans, but they were hardly worth the labor.

Оню, Linden Heights. E. B. Champion.—Beans yielded fully one-half more than untreated. The green beans carried the largest-sized pods I ever saw, but the yield was not increased so enormously as in the case of the wax beans. In this case the increase was so marked as to cause wonder among my neighbors.

Pennsylvania, Cresson. V. P. Sanker.—On ground which never before would raise a crop of beans, had marvelous crop this year, the heaviest ever raised in this locality. Planted seven rows in middle of field without inoculating, and the old conditions prevailed.

Northeast. John Wheeler.—Result of inoculation splendid. Refugee beans for canning factory. One-third acre yielded \$50 to \$60 clear profit. I think it

can not be beat by use of fertilizer.

RHODE ISLAND, Kingston. H. J. Wheeler, director, Rhode Island Agricultural Experiment Station.—Concerning the wax beans and green-podded bush beans, both are continuing to show very striking benefits from the use of the inoculating material, so much so that I think it would be a very important matter, economically, if one were growing them on a large scale, whether the land was inoculated or not.

Vermont, Middlebury. J. E. Sperry.—Gain from inoculation, 11 bushels per acre over seed not treated, planted side by side. There is no doubt but that it is a

great help.

# Soy Beans.

Alabama, Rash. W. W. Lee.—All inoculated but six rows. Inoculated began showing result of inoculation in a few days after they came up, and harvested 50

per cent more than the other.

Georgia, Gainesville. John E. Miller.—The soy-bean inoculation I got last spring was a complete success. I planted 10 or 12 acres on an old barren field, and they are from 12 to 36 inches high. Have not found a single one that was not inoculated. One had tubercles 26 inches from the base. I think your Department a great help to the farmers.

Kentucky, Winchester. Dr. M. S. Browne.—Twelve thousand five hundred pounds dry hay, ready for storing, per acre; ground where seeds were not inoculated at rate of 1,500 pounds cured hay per acre. Soil, medium bluegrass sod. Non-inoculated, a failure; inoculated, a wonderful crop. Date of planting, April

15; date of harvesting, July 25.

Maryland, Bynum. Wilmer P. Hoopes.—Our soy beans drilled in with corn in rows 3½ feet apart, the whole crop making about 20 tons of silage per acre. The beans just covered the space between rows and yielded at least 2 tons per acre. The roots were just covered with nodules.

- Missouri, Marionville. U. L. Coleman.—Where inoculation was used the beans did a great deal better and produced fully one-third more beans. I found no nodules on the soy beans where not inoculated. The inoculation was a success.
- New York, Maryland. W. W. Stead.—There was about 75 per cent gain over a piece of ground of the same size which was not inoculated. I think that the inoculation was a great success.
- Virginia, Carysbrook. C. E. Jones.—All of the inoculated hills showed an abundance of nodules, while only a total of four were found on the uninoculated ones, notwithstanding the proximity of the inoculated seed, the roots of both plants often interlacing. One row inoculated by culture and one by soil from soy roots having numerous nodules showed an equal number of nodules; the check had none. I find that the roots show far more nodules than I have ever seen before, and this development seems more excessive on the poorer parts of the field
  - Simplicity. Mrs. Rose Fisher.—Nearly all plants had from 1 to 29 large nodules, nearly all located on the taproot about 1 to 2 inches in the ground. An adjoining field, not treated, showed but very few nodules.

# Hairy Vetch.

- Alabama, Tuskegee. George W. Carver, director, Agricultural Experiment Station.—
  The inoculated plot grew vigorously—in fact, made an enormous growth—and made 7 bushels of seed to the acre. The other was so small that I did not thrash it out.
- Kentucky, Trenton. Phil. E. Bacon.—Used vetch material with best results. The growth was very heavy and the roots as full of nodules as any illustration I have ever seen, some clusters fully as large as the end of my finger.
- Mississippi, Aberdeen. Isaac H. Hunt.—Inoculated was better in every way than the untreated seed. We are very much encouraged by what we have already seeded.
- Nebraska, Taylor. Ray G. Hulburt.—Bloomed three weeks earlier; more seed; larger plants. Oats sowed with it were larger. Roots of vetch plants were crowded with tubercles, single and in masses. Sowed too close; germs spread to untreated part in July, but it never caught up. Some plants 10½ feet long.
- New York, Butterfly. J. E. Baker.—Fine growth on very poor soil. On a high, dry, gravelly knoll grew 6 to 8 feet and a mass of blossoms and pods. Have never succeeded in growing anything on this piece before.
- Washington, Seattle. David B. Porter.—Last fall I treated winter vetches with the solution prepared as directed and planted the same broadcast over a small patch of ground with a good deal of clay, some blue and some shot clay. On turning the ground over in the spring, there was a network of roots forming a thick sod about 8 or 10 inches deep and very heavily charged with the nitrogen nodules, some roots having as many as 40 or 50. I have used other rotted vegetable matter with this to form a humus and have now a fine friable soil which yielded very heavily this year.
- Wisconsin, Germania. C. E. Pierce.—The benefit was very plain, promoting a rank growth, adding at least one-third to the crop.
  - Meadow Valley. C. H. Johnson.—Inoculation successful. Nodules in quite large clusters on lower fibers of the roots, more scattering near the surface. Planted on high sandy land.

#### Crimson Clover.

Alabama, Tuskegee. George W. Carver, director of Agricultural Experiment Station.—This was quite noticeable, that on the adjoining plot the stand was just as good as on the inoculated plot, but it grew very poorly. It remained small

and yellow throughout the season. The inoculated plot grew fairly well and was very rank and green in color. These plots were treated in every way alike, except in the matter of inoculation. One end of the inoculated plot did not get any of the inoculating material, and the small, inferior clover was very noticeable.

Prinsylvania, Bellefonte. James A. B. Miller.—Fair catch on thin soil. About 6 inches high. Failure on same soil last year without inoculation. Seems thrifty

and gives every promise of successful catch.

Joanna. H. E. Plank.—It is a satisfaction to inform you that there was a much greater mass of fibrous roots on the plants grown from the seed treated with the material than on the plants from the untreated seed. The nodule formations are much more abundant on the former class of plants. There is a good stand of clover.

Washington, Spokane. Henry M. Richards.—The results heretofore with the same amount of seed have been a very stunted growth and scant blooming. The seed prepared with the inoculating material has produced a most luxuriant growth and a perfect mass of bloom, an improvement so great that it is difficult to describe.

West Virginia, Elm Grove. George Fox.—Seed inoculated 50 per cent superior to

the seed which was not inoculated.

### Sweet Peas.

California, Los Angeles. W. L. Cleveland.—The seeds were treated in accordance with the instructions you sent me and then planted in the usual manner. The result of this seeding was a hedge of vines that grew to a height of about 8 to 10 feet, covered with a lot of fine, large blossoms that were the delight of the whole neighborhood. Across the street, and treated in the ordinary way with the same seed that I furnished them but without the inoculation, the vines scarcely grew 5 feet and the flowers were small and few. I consider the thing a success.

Massachusetts, West Roxbury. F. G. Floyd.—Plants were very luxuriant and about 12 feet high. Leaves very large and rotund; flowers very large and of fine color. Plants produced several double flowers—i. e., having two or three

entire or partially formed standards.

New Jersey, Newark. William J. Hesse.—The crop was a complete success, while other growers in this location did not succeed at all. While I have no record of the quantity of the crop, I will say that I had a larger crop, better blooms of lasting quality than any other grower with the same amount of ground planted. I had two awards at the New Jersey Horticultural Society for these same blooms in June and July at Orange, N. J., and I know that had it not been for the inoculating of the seed I would not have been so successful.

### Canada Field Peas.

California, Colton. M. S. Ratliff.—By actual experience peas inoculated made fully one-half better growth than peas not inoculated. These peas were sown in an

orange grove for green manuring.

Riverside. W. H. Backus.—I found bacteria everywhere; every spadefull, wherever test was made, showed bacteria on the fibrous roots, some at a depth of over 12 inches. I believe it is one of the best discoveries for the horticulturist yet found. I was not a believer at first in the field pea, and dug up vines every year for the last four years looking for bacteria. It took two or three years to get the bacteria in quantity. Now, with the culture sent out, if it is properly made up and the seed inoculated, one can get the bacteria at

one planting, the amount depending on the care, etc., and the growth is so dense that common weeds have no show whatever. Humus is getting down to nothing on commercial fertilizers. I have an old orange grove, once the best in this section, but it has been going back in quality and quantity the last four years on liberal applications of commercial fertilizers. I have watched green manuring on this account, and wherever the field pea has been planted for a series of years the improvement is remarkable; where bur clover has been allowed to grow during the winter and plowed under in the spring, the improvement is quite noticeable, as on my own place. I see no way so good and cheap as inoculating the soil and sowing peas or other legumes. Fertilizers are expensive, too stimulating, and when withheld for a year or two the grove goes back rapidly. I believe if young groves were planted to peas right from the start the soil would approximate to a virgin soil for years, if a little phosphoric acid and potash were added from year to year as needed. If one could save from \$20 to \$25 per acre every year on his fertilizer bills, it would foot up a grand total for the entire section.

- Maine, Auburn. G. L. Thomas.—The product out of No. 1 strip without any fertilizer was as much as out of No. 3 with the heavy manuring. In other words, the inoculating culture had done as much for strip No. 1 as the barnyard dressing had done for No. 3, while No. 2 (inoculated and manured) had produced as much as the other two strips combined. The growth in No. 2 was excessively strong and luxuriant, and this was due to the nitrogen drawn from the air by the vaccinating cultures. No. 1 was fair yield and cost about 60 per cent as much as No. 2 and about 47 per cent of that for No. 3.
- Pennsylvania, Hartstown. J. T. Campbell.—Where soil was inoculated the result was marvelous—four times as great as where there was no inoculation. Nodules one-half inch in diameter.
- Texas, Keene. A. P. Wesley.—Nodules formed on vines when quite young and the growth was fine, while the land they were planted on was worn-out clay. I think it a success.
- Wisconsin, Bay City. Chicker Brothers.—A very satisfactory crop was raised where failure had attended for seven years.

### Velvet Beans.

- FLORIDA, Jacksonville. E. H. Armstrong.—Thirty to 50 per cent increase over that where seed was not inoculated with the velvet-bean culture; same for cowpenseson dry, somewhat unfavorable.
- Louisiana, Cades. C. E. Smedes.—Increased the nodules and the vines 30 per cent. Vines were plowed under.

### Berseem.

California, Berkeley. David Fairchild.—You will be interested to know that at Berkeley this year there was an immense difference between the plots of berseem from treated and untreated seed, the former being several hundred percent better than the latter.

#### Peanuts.

VIRGINIA, Poplarmount. Charles Denney.—Inoculated a piece of land according to your instructions, and planted Spanish peanuts. Increased yield at the rate of 5 bushels per acre.

### Miscellaneous.

Pennsylvania, Lockhaven. George P. Singer.—I used them in my botany and naturestudy classes in this way: I furnished each student with a number of pots of fine white sand. The same day they planted beans and clover, and also the same kind of seeds inoculated with the bacteria. Each pot was exposed to the same conditions and the inoculated compared as to growth with the uninoculated. There was no especial difference in germination, but when the plants had put forth their first leaves the ones inoculated began to grow much faster than their neighbors. It was not long until they were twice as high, and while the ordinary seeds produced plants stunted and ill-nourished, the inoculated seeds in many cases produced a large bean stalk with fully developed pods and beans. The clover seed showed the same result. Root nodules were formed in great abundance. All in all, it was the most interesting experiment I have ever tried in my classes, and it aroused a great deal of interest in the students. I am confident that if clover and beans will grow as they did for us in sand which was quite free from organic matter, your nitrogen-fixing bacteria will solve many problems for the intelligent farmer.

NORTH CAROLINA, Gibson. Dr. N. M. McLean. - As to "nodule formation," a test was made by myself in person to determine this feature. Sterile soil (obtained from a sand subsoil several feet below the surface), to which was added a certain amount of phosphoric acid and potash, obtained from acidulated rock and muriate of potash, were placed in one-half gallon pots. Each legume tested was planted in a number of these pots. To a certain number a small quantity of the "inoculating material" was added, with others as "control pots." In each test a marked contrast was noticeable in a short time, the inoculated pots showing several times the plant growth that the control or uninoculated pots did, and in each case the inoculated pots showed a plentiful supply of nodules on the plant roots. An experiment on a large scale was then tried. A trench 3 feet broad and 12 feet long was dug out 30 inches deep. This was in a heavy clay-loam soil. The trench was filled with this same sterile soil used in the pots fertilized with the phosphoric acid and potash. In each square (3 feet by 3 feet) a legume was planted—alfalfa, crimson clover, soy beans, and velvet beans. Each variety of seed was inoculated with material you so kindly furnished, and in each test there was an abundant "nodule formation." In each one of these several tests the control pots and plots verified the results beyond the possibility of doubt. I hope next season to be in a position to make a tabulated report that may be of use to others. As to myself, I consider your discovery the greatest one of the age and hope you may live to see a universal acknowledgment of the same.

## SUMMARY.

Owing to the direct effect of the nodule-forming bacteria upon legumes, these plants are supplied with a source of nitrogen not available to most other plants. Consequently, the legumes can flourish in a soil practically devoid of nitrogen.

The effect of legumes upon succeeding crops of any kind is beneficial, because of the fact that the soil is enriched rather than impoverished

by these plants.

Where nitrogen-fixing bacteria are lacking, it is possible to introduce them artificially either by transferring soil from an old field where the desired leguminous crop has been successfully grown, or by the use of pure cultures of the proper organism.

The method of transferring soil is objectionable because of the inconvenience and expense, and is apt to be dangerous on account of

the possible transfer of weeds, insect pests, and plant diseases.

The use of the German preparation, nitragin, has not been a success, probably owing to the method of growing and distributing the bacteria.

In order to increase or maintain the virulence of nodule-forming organisms, they must be cultivated upon nitrogen-free media. Growth upon rich nitrogenous media tends to diminish and frequently destroys the nitrogen-fixing power, since this element can be obtained more easily from the medium than from the air.

Various external conditions, such as temperature, moisture, acidity, amount of nitrogen in soil, etc., all have a direct effect upon the legume bacteria, and the failure of nodules to develop may often be traced to such a cause.

The nitrogen is fixed by the bacteria in the nodule and becomes available by the action of the plant in dissolving and absorbing the combined nitrogen in these organisms.

The nodule-forming organism is a true micro-organism, and there is but one species, namely, *Pseudomonas radicicola* (Beyerinck) Moore. The difference in the infective power of bacteria from different host plants is due to slight physiological variations which can be broken down readily by artificial cultivation. Quicker and surer results, however, are obtained by preparing separately cultures for each species of legume.

It is possible (in rarely observed cases) for nitrogen-fixing bacteria to penetrate the roots of plants and be of decided benefit without the formation of nodules or any external evidence of their presence.

While it is desirable that artificial inoculation be made at the time of planting, experience has shown that under certain conditions crops of several years' standing are improved by adding bacteria to the soil.

Inoculation is usually of no benefit to soil already containing the proper bacteria, although there may be exceptions. It need not be practiced where the soil is already rich in nitrogen, because, in such soils, nodules are formed with difficulty and are of little benefit. Furthermore, the use of such soils for growing legumes is generally a mistaken policy, especially if grown for green manuring.

The inoculation of seed and soil by means of pure cultures grown and distributed according to methods devised by the Department of Agriculture is shown by the reports of practical farmers to be of distinct advantage when used under circumstances that will permit benefit.